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The World Hypertension Day (WHD) was first inaugurated in May 2005 as an initiative of the World Hypertension League (WHL) and has become an annual event ever since (this year on May 17). The purpose of the WHD is to promote public awareness of hypertension and to encourage citizens of all countries to prevent and control this silent killer and the modern epidemic.

Yugoslav League of Hypertension (from 2008 Serbian Society of Hyprtension) has been a member of the WHL since 1996. Today, Serbian Society of Hypertension is affilated society of the International Society of Hypertension.

Svetski dan borbe protiv hipertenzije (*World Hypertension Day* - WHD) ustanovljen je u maju 2005. godine na inicijativu Svetske lige za hipertenziju i od tada se obeležava svake godine u maju (ove godine 17. maja). Cilj WHD je podizanje svesti stanovništva svih zemalja sveta o hipertenziji i značaju prevencije i kontrole tog „tihog ubice" i savremene epidemije.

Jugoslovenska liga za hipertenziju (od 2008. godine Udruženje za hipertenziju Srbije) član je Svetske lige za hipertenziju od 1996. Danas je ovo Udruženje pridruženi član Internacionalnog društva za hipertenziju.

ORIGINAL ARTICLES

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Comparison of diagnostic criteria in patients with amyotrophic lateral sclerosis – the contribution of electromyographic findings

Poređenje dijagnostičkih kriterijuma kod bolesnika sa amiotrofičnom lateralnom sklerozom – doprinos elektromiografskih nalaza

Aleksandra Dominović-Kovačević*, Duško Račić*, Sanja Grgić*, Zoran Vukojević*, Sladjan D. Milanović†, Tihomir V. Ilić‡§

University of Banja Luka, University Clinical Centre of the Republic of Srpska, *School of Medicine, Republic of Srpska, Bosnia and Herzegovina; University of Belgrade, †Institute for Medical Research, Belgrade, Serbia; University of Defence, ‡Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Military Medical Academy, §Clinic of Neurology, Belgrade, Serbia

Abstract

Background/Aim. Diagnosis of amyotrophic lateral sclerosis (ALS) is based on combination of clinical signs and electrophysiological correlates of pathological process which takes place in general. New electrophysiological criteria Awaji-Shima (AS) additionally qualify the complex fasciculations and neurogenically modified potentials of motor units as signs of active lesions of peripheral motor neuron, contrary to previously valid revised El Escorial criteria (rEE). The objective of this research was to determine the clinical significance and advantages of using the AS criteria in patients with ALS. **Methods.** Thirty patients (59.2 ± 10.9 years, 57% of them with spinal form of the disease) with clinically suspected ALS were monitored from the time of diagnosis until reaching the category of definitive diagnosis or death. The clinical evaluation and electromyographic (EMG) examinations were carried out at 3-month intervals. **Results.** By applying the AS criteria, the category of probable or definite diagnosis was achieved in all patients with ALS, except in one (96.6%), as contrary to the rEE (33.3%), after 6 months of the follow-up period. The subclinical affection in more than two body regions has been defined through detection of denervation potentials (80% of the patients by using the AS, or 67% by the rEE criteria). The complex fasciculations were registered particularly often in small muscles of the feet (37–40%). **Conclusion.** Application of the AS criteria improve the achievment of category of probable or definite diagnosis of ALS by 2.7 months earlier compared to the rEE. This outcome is particularly affected by a higher frequency of positive EMG findings, when the AS criteria were employed. Early determination of diagnosis provides the better perspective and more frequent participation of the ALS patients in pharmacotherapy studies intended to establish new therapeutic options.

Key words:
amyotrophic lateral sclerosis; electrophysiology; electromyography; predictive value of tests; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Dijagnoza amiotrofične lateralne skleroze (LS) zasniva se na kombinaciji kliničkih znakova i elektrofizioloških korelata patološkog procesa koji se odvija u osnovi. Novi elektrofiziološki kriterijumi, Awaji-Shima (AS), dodatno kvalifikuju kompleksne fascikulacije i neurogeno izmenjene potencijale motornih jedinica kao znakove aktivnih lezija perifernog motornog neurona, nasuprot ranije važećim revidiranim El Escorial kriterijumima (rEE). Cilj ovog istraživanja bio je da se utvrdi klinički značaj i prednosti primene AS kriterijuma, kod obolelih od ALS. **Metode**. 30 bolesnika (59,2 ± 10,9 godina, od kojih 57% sa spinalnom formom bolesti) sa klinički suspektnom ALS, praćeno je od vremena postavljanja dijagnoze do postizanja kategorije definitivne dijagnoze ili smrtnog ishoda. Kod bolesnika su obavljane klinička evaluacija i elektromiografski (EMG) pregledi u tromesečnim intervalima. **Rezultati.** Primjenom AS kriterijuma, nakon 6 meseci praćenja, kategorija verovatne ili pouzdane dijagnoze postignuta je kod svih bolesnika sa ALS, izuzev jednog (96,6%), nasuprot rEE kriterijuma (33.3%). EMG nalazi bili su pozitivni u ≥ 2 telesna regiona kod 80% bolesnika primenom AS, odnosno 67% primenom rEE. Kompleksne fascikulacije zabeležene su posebno učestalo u malim mišićima stopala (37–40%). **Zaključak.** Primenom AS kriterijuma kategorija vjerovatne ili pouzdane dijagnoze ALS postiže se za 2,7 meseca ranije, u poređenju sa rEE, na šta

Correspondence to: Tihomir V. Ilić, University of Defence, School of Medicine of the Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 11 3609064. E-mail: tihoilic@gmail.com

posebno utiče veća učestalost pozitivnih EMG nalaza. Ranije utvrdivanje dijagnoze donosi perspektivu veće zastupljenosti obolelih u farmakoterapijskim studijama sa novim terapeutskim agensima.

Ključne reči:
amiotrofijska lateralna skleroza; elektrofiziologija; elektromiografija; testovi, prognostička vrednost; osetljivost i specifičnost.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disease caused by the selective damages of motor neurons of the cerebral cortex, brainstem and anterior horns of the spinal cord. The disease is clinically manifested by the combination of lesions of the central (CMN) and peripheral motor neuron (PMN) with progressive development of muscle weakness and atrophy [1]. The diagnosis of ALS is based on the combination of neurological findings and subclinical signs of PMN lesions, which are revealed by electromyography (EMG) [2].

During the evolution of diagnostic concept in ALS, several diagnostic approaches were applied, starting with Lambert criteria [3]. Over time, a concept of diagnostic categories divided into suspected, probable and definite diagnosis of ALS has been established. Such probabilistic approach is, on one hand, based on a broad differential diagnosis of ALS, which requires high specificity, and on the other hand, on the fact that there is no biological surrogate marker of the disease which would facilitate the diagnosis.

Furthermore, therapeutic options in the treatment of this disease are extremely modest, with a slight exception of a beneficial effect of the antiglutamatergic drug riluzole which affects the length of survival mainly in the categories of probable and definite ALS [4]. Therefore, effective testing of the innovative pharmacological agents, with highly probable diagnosis as imperative, is limited to a small proportion of patients. In this way, the possibility of accepting incorrect diagnosis in the process of testing new drugs is substantially reduced and high credibility of the findings is ensured.

However, by using the restrictive criteria (revised El Escorial – rEE criteria) based on a broad differential diagnosis of ALS, it is easy to understand that the sensitivity is significantly reduced [5, 6]. Therefore, in order to facilitate the diagnosis, in 2006 there was a modification of the rEE criteria, which were proposed to be called Awaji-Shima after the place of the meeting. New criteria, particularly emphasized the phenomena of complex fasciculations and neurogenically modified motor unit potentials which can be detected and analyzed by using the needle electromyography. These indicators, especially the complex fasciculations, as it is suggested, represent the electrophysiological correlates of acute denervation or additional markers of affected PMN. In that way, the number of EMG indicators increases, which in combination with the clinical signs of PMN lesions, increases the sensitivity of Awaji diagnostic criteria [7].

This research compared the difference between two systems of classification by using the rEE and Awaji-Shima criteria in relation to the time required to achieve the category of probable and definitive diagnosis of ALS, and the time of initial evaluation on the reasonable suspicion of ALS (category of suspected diagnosis). In addition to that, the study separately discussed the subclinical aspects of affected body regions based on the evaluation of electromyographic findings and their impact on the achievement of specific categories of the disease according to either the rEE or Awaji-Shima criteria and relation to the time course.

The primary objective of the study was the time necessary to determine the category of probable or reliable diagnosis of ALS, expressed in months.

The secondary objective was the analysis of denervation potential frequency - the complex fasciculation in different body regions at first place.

Methods

Patients

Sixty-eight patients with clinical presentation of progressive muscular weakness suggestive for ALS were examined at the Clinic of Neurology, University Hospital Clinical Centre Banja Luka, the Republic of Srpska, Bosnia and Hercegovina in the period 2012–2014. The suspected ALS was diagnosed in 30 patients (20 men, average age of 59.2 ± 10.9, ranging between 39–75 years). The analysis excluded patients with different forms of neuropathy (primarily those with multifocal motor neuropathy) and the patients with positive family history of motor neuron disease since the progression of sporadic and familiar forms of the disease are not the same. Demographic data of patients is presented in Table 1.

Table 1

Demographic and baseline clinical characteristics of patients with amyotrophic lateral sclerosis (ALS) at study entry

Parameters	Total sample (n = 30)	Spinal form (n = 17)	Bulbar form (n = 13)	*p*
Age (years), mean (SD)	59.2 (10.2)	54.8 (10.8)	64.9 (8.8)	0.01
(range)	39–75	39–71	45–75	
Gender (female)	10	2	8	
Time elapsed from symptom onset (months), mean (SD)	10.2 (4.5)	9.6 (4.8)	10.9 (4.0)	n.s.
(range)	3–18	5–18	4–18	
ALS-FRS, mean (SD)	43.3 (3.6)	41.8 (4.2)	46.1 (3.9)	n.s.

***ALS-FRS – ALS Functional Rating Scale; SD – standard deviation; n.s. – not significant.**

When they entered the study, the patients were subjected to clinical evaluation and electromyographic examination at 3-month intervals, either to achieve a category of definite diagnosis or death, if it occurred before reaching this category.

At each visit to the Clinic, on the basis of the neurologic findings and the EMG examination, currently affected body regions in patients were defined (bulbar region, cervical, thoracic and lumbosacral spinal region) according to clinical signs of CMN and PMN lesions as well as the EMG correlates of the PMN lesions. Based on that evaluation, the patients were categorized in accordance with the rEE and AS criteria (Table 2). Additionally, the assessment of functional score (FS) at each examination and for each patient was made using The Amyotrophic Lateral Sclerosis Functional Rating Scale (ALS FRS).[8]

Electromyography

EMG was done with concentric disposable electrodes (TECA, 25 mm × 30G) on the equipment model Medelec Synergy (VIASIS, United Kingdom) by the same researcher under the same environmental conditions (the temperature of extremities 30°C), for muscles in the mentioned body regions, according to the selection as follows: bulbar region – one muscle [*m. mentalis – mMEN* (*n. facialis*)]; cervical spinal region – three muscles [*m. deltoideus – mDEL* (C5-C6*; n. axillaris*), *m. biceps brachii – mBB* (*C5-C6; n. musculocutaneus*) *and m. abductor digiti minimi – mADM* (*C8-Th1; n. ulnaris*)]*;* thoracic spinal region: muscles in several segments [*mm. paravertebrales toracales – mPV* (Th2–Th10)]; lumbosacral spinal region – two muscles [*m. vastus lateralis – mVL* (L2–L3–L4; *n. femoralis*), *m. tibialis anterior – mTA* (L4–L5–S1; *n. peroneus*), *m. extensor digitorum brevis – mEDB* (L5–S1; *n. peroneus*) *and m. flexor hallucis brevis – mFHB* (L5–S1; *n. plantaris medialis grana n. tibialis*)].

The signs of PMN lesion were considered to be the occurrence of a denervation potential type of fibrillation, positive sharp waves (PSW) and fasciculations if they were found in the target muscle in two or three locations during the relaxation. The neurogenic lesion was also indicated by the presence of changed motor unit action potential (MUAP) on the increase of amplitude pattern, extended duration and increased number of phases (poly-phases) and with the reduction of an interference pattern. The myotomal distribution was confirmed if the electrophysiological signs of the PMN lesion were registered in the muscles that were differently innervated by the spinal and peripheral nerves.

Statistical analysis

The data were analyzed by the method of descriptive and analytical data processing (Mann-Whitney U test, Chi-square test, Fisher's exact test, Wilcoxon signed-rank test).

The time necessary for the achievement of the specific diagnostic categories of ALS was correlated with a number of EMG positive regions and the pattern of diagnosis (bulbar vs. spinal form) whenever the McNemar test was used. The results were presented as means ± standard deviation. Statistically significant differences were considered if p-value < 0.05.

Statistical analysis was done using the application for the data processing "SPSS" version 16.0, SPSS Inc., USA.

Results

Initial clinical and electrophysiological examination included 68 patients whereby the diagnosis of suspected ALS was confirmed in 30 patients. In other patients who were initially examined, the multifocal motor neuropathy was diagnosed in 5 patients, polyradiculopathy in 24 patients, paraneoplastic neuropathy in 4 patients, spinal muscular atrophy in 3 patients and myositis in 2 patients. The initial clinical presentation of the spinal form of ALS was present in 17 (57%) patients. Comparing the forms of disease in the tested sample, it was found that the patients with the bulbar form of the disease were about 10 years older (64.9 ± 8.8 vs. 54.8 ± 10.8; $p = 0.010$) (Table 1).

Table 2

Comparative overview of the distribution and affected number of body regions necessary to diagnose the categories of amyotrophic lateral sclerosis (ALS) in accordance with rEE and AS criteria

Parameters	rEE criteria	AS criteria
Possible ALS		
CMN	1	1
PMN	1*	1
Probable – laboratory supported ALS		
CMN	1	
PMN	1*	
Probable ALS		
CMN	2	2
PMN	2*	2
Definite ALS		
CMN	3	
PMN	3	

CMN – central motor neuron; PMN – peripheral motor neuron; *The level of PMN lesion must be rostral in relation to CMN lesion along the neural axis (neuraxis); rEE – revised El Escorial criteria; AS – Awaji-Shima criteria.

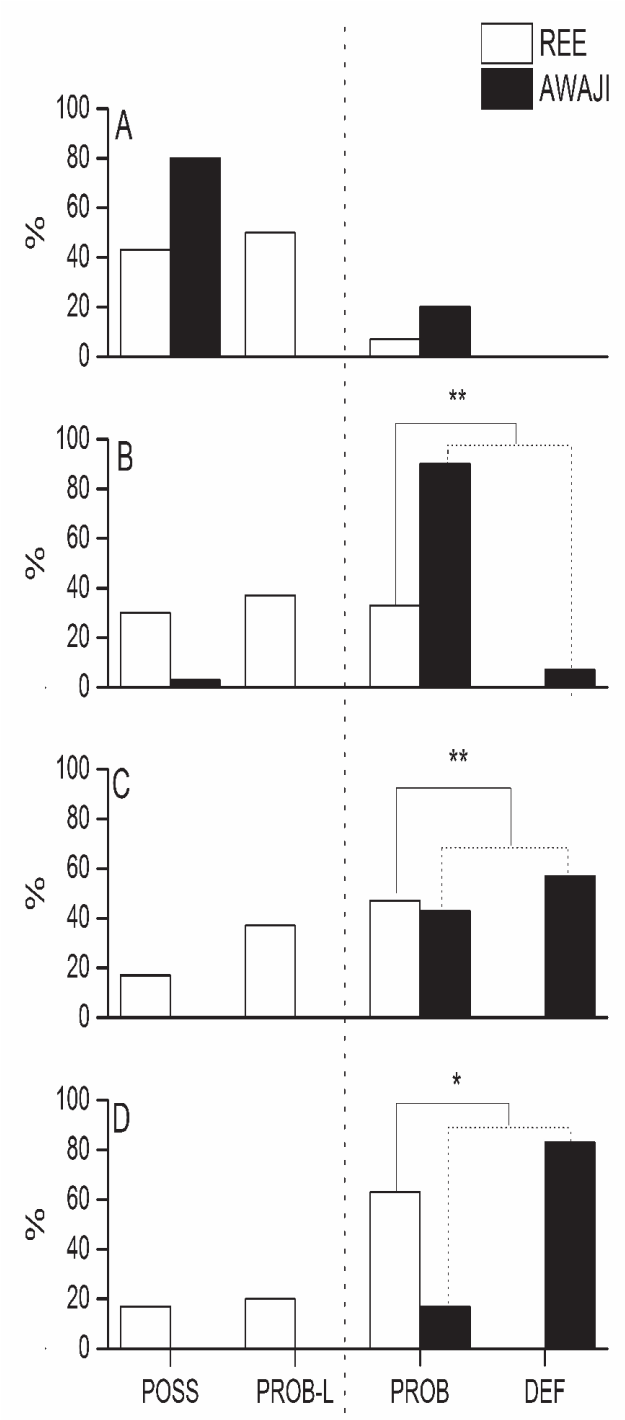

Fig. 1 – Distribution of diagnostic categories by using the rEE or AS criteria according to the time intervals in which repeated evaluations were done (A – 3 months; B – 6 months; C – 9 months; D – 12 months).
***** $p < 0.05$, ****** $p < 0.01$ (indicates the level of statistical significance); rEE – revised El Escorial criteria; AS – Awaji-Shima criteria; POSS – possibly; PROB – probably; DEF – definitely.**

Body regions in which the initial difficulties in the spinal form of the disease were expressed, were evenly represented – there were 9 patients with the cervical and 8 with the lumbosacral clinical presentation.

During the period of monitoring, 7 out of 30 patients diagnosed with ALS died in the interval between 10 and 14 months after the first examination, so, after 12 months, it was possible to analyze 26 patients in total, and after 15 months, only 23 patients.

According to the data obtained from an anamnestic interview, the patients (> 2/3 examinees) took the first examination in the period between 6 and 24 months from the time of the subjective perception of symptoms, which were subsequently confirmed as the correlates of lesions of the CMN and/or PMN (Table 1).

The primary objective of the study, the time necessary to achieve the category of probable or definite diagnosis of ALS, was achieved in all patients in the period between 9 and 15 months of monitoring, or after 4 or 6 repeated examinations (including the initial). By using the rEE criteria, the average period necessary to achieve the category of probable and definite diagnosis was 9.4 months, while for the AS criteria, it was 6.7 months which created a difference of 2.7 months.

Accordingly, the target diagnostic categories (probable or definitive) by using rEE and AS criteria was achieved at different rates at successive time points as shown in Figure 1 and Table 2. The key difference was generated in the second control examination (6 months after the initial evaluation), when applying rEE category probable (or definitive) diagnosis was achieved only in 1/3 of patients, as opposed to 96.6% when AS criteria were applied. This was also the key findings of the study.

However, when we analyzed the time required to achieve the target diagnostic categories in relation to the disease form (bulbar vs. spinal), the key difference was detected on the third examination (6 months after the initial one) showing that the AS reached the target for all ALS patients with bulbar form compared to less than half patients with spinal form (46.2%) (Figure 2).

In an attempt to define the contribution of number of positive body regions, in which the contribution in the detection of PMN lesions was achieved by using the EMG for diagnostic categories of probable and definite diagnosis, there were distinguished findings – at the third examination (after 6 months) of the patients classified by using the rEE criteria, 67% had positive EMG findings in 2 or more body regions, in contrast to 80% of patients classified by the AS criteria ($p = 0.031$) (Figure 3). A similar difference was determined by a number of patients, who at the fourth examination (in the ninth month of monitoring) showed the positive EMG findings in three or more body regions according to the used criteria (rEE = 42% vs. AS= 71%, $p < 0.05$) (Figure 3).

The denervation potentials (fibrillation, PSW and complex fasciculations) were present in the patients with ALS with different frequency. The complex fasciculations were particularly frequent in small foot muscles, mEDB and mFHB (40% and 37% respectively) at the third examination (in the sixth month of monitoring). Contrary to that, fibrillation and PSW were registered in arm and hand muscles (*mDEL*, *mBB* and *mADM*) as well as in the paravertebral musculature of thoracic region (*mPV*) more frequently (Figure 4).

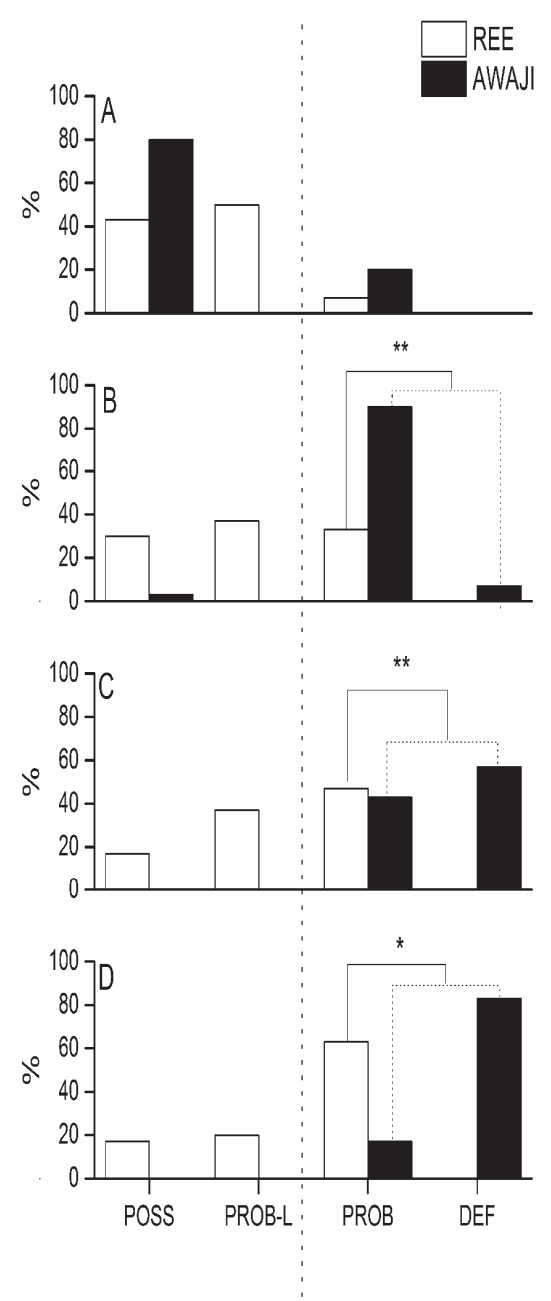

Fig. 2 – Distribution of diagnostic categories by using the rEE or AS criteria according to the forms of the disease, spinal vs. bulbar according to the times of evaluation.
Marks are the same as in Fig. 1 (A – 3 months; B – 6 months; C – 9 months; D – 12 months), so as abbreviations (POSS – possibly; PROB – probably; DEF – definitely).

Finally, we tried to determine whether the degree of functional deficits measured by the FS in the patients who achieved the criteria of diagnosis by using the rEE or AS was different, which proved to be true only for the study "breakpoint" – the third examination at the sixth month of monitoring when the FS median of the AS was 34, or 30.5 of the rEE ($p = 0.017$).

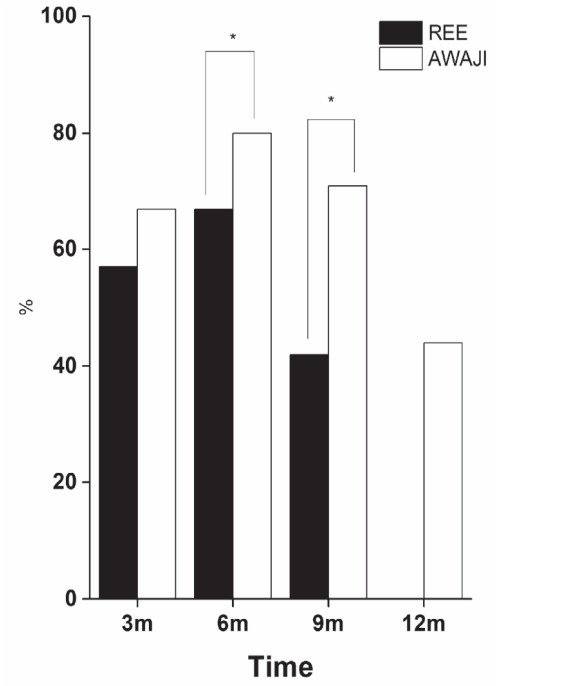

Fig. 3 – Representation of affected 2 or more body regions, in the categories of probable and definite diagnosis of ALS and according to positive EMG findings as correlates of PMN lesions. The abscissa includes the times of evaluation in months.
***p < 0.05 (indicate the level of statistical significance); REE – Revised Ed Escorial criteria; AWAJI – Awaji-Shima criteria.**

Discussion

The key finding of this prospective study refers to the time required to make a definite diagnosis of ALS, which, by using the AS criteria, is shortened on average for less than 3 months. By using the AS criteria the definite diagnosis is made on average 9.81 ± 1.81 months from the initial examination, while in the period of 12 months from the initial examination none of the patients were diagnosed with definitive ALS by using the rEE criteria. The difference between this study and previous similar ones refers to the absence of monitoring the evolution and contribution of the criteria during the longitudinal observation of patients.

Namely, the diagnosis of amyotrophic lateral sclerosis significantly complicates the complex clinical picture with numerous overlappings clinical presentations with other diseases of the peripheral motor neuron as well as the absence of a reliable biological marker. Therefore, the diagnostic protocol requires both the clinical and electrophysiological signs of the disease, which eventually resulted in three (AS) or four (rEE) categories of diagnosis among which the reliability increases with a number of body regions.

Therefore, significant acceptance of electrophysiological signs of denervation activity extends the possibility of determining the affected body region from which the findings arose in our and similar studies.

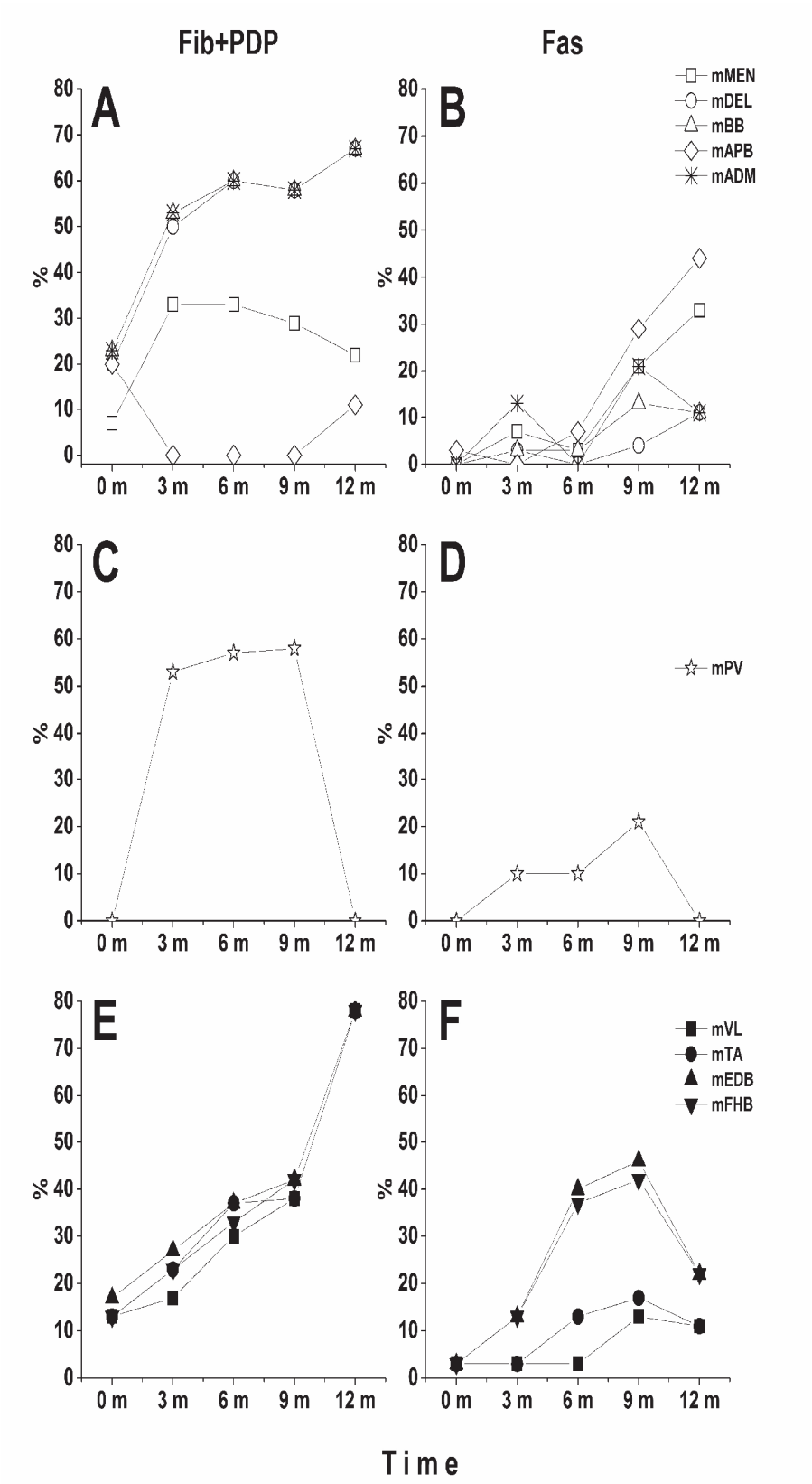

Fig. 4 – Temporal and spatial distribution of the frequency of denervation activity in patients with amyotrophic lateral sclerosis.
Fib – fibrillation; PDP – progressive denervation; FAS – fasciculations.
mMEN – ***m. mentalis;*** **MDEL –** ***m. deltoideus;*** **mBB –** ***m. biceps brachii;*** **mAPB –** ***m. abductor pollicis trevis;***
mADM – ***abductor digiti minimi;*** **mVL –** ***m. rastus lateralis;*** **mTA – m. tibialis anterior; mEDB –** ***m. extensor digitorum brevis;***
mFHB – ***m. flexor mallucis brevis.***

The findings in our study are slightly weaker in comparison to the study of Okita et al. [9], since in their study the disease was diagnosed about 6 months earlier by using the AS criteria, but if the time was measured from the onset of symptoms. This difference may be partly due to the known effects of a relative delay between the onset of symptoms and refers to the tertiary center [10] in our study (Banja Luka, the Republic of Srpska), which was 10.2 ± 4.5 months.

Furthermore, after 3 examinations (6 months after entering the study), 28 of 30 patients (96%) met the AS criteria for probable or definite diagnosis, contrary to 33.3% when applying the rEE criteria. Also, using the AS criteria did not result in the loss of specificity, while the sensitivity was improved in categories of probable and definite diagnosis of ALS to 6.7% in the third month by using the rEE, contrary to 20% by using the AS. Moreover, the AS criteria provided the sensitivity of 100% for the mentioned above diagnostic categories in the ninth month of the monitoring, while the sensitivity according to the rEE in the twelfth month was only 60%. This is consistent with findings of de Carvalho and Swash [11] in one of the first publications dealing with similar issue, but at the same time, which is significantly better than some other studies [12, 13]. However, regarding the two last studies, it is necessary to emphasise that it is difficult to make the direct comparison due to the methodological differences; the study of Noto et al. [12] was about an ethnic group of Asians, while in the study of Boekestein et al. [13], the design was a retrospective study. In addition to that, fewer muscles were examined electromyographically, which could certainly influence the sensitivity of the approach.

The assumption that a set of the AS criteria is particularly sensitive to defining the category of the disease in the patients with bulbar form [12–14] was not confirmed in our case probably due to the relatively small number of examinees, since only 13 patients with ALS suffered the bulbar form of the disease.

In terms of detecting the denervation activity in general, the key point in the AS approach refers to the acceptance of specific types of the fasciculations, which are complex and unstable, as equivalent to current denervation changes [14]. However, it is necessary to emphasize that the fasciculations, at the same time, are not the specific occurrence in ALS, but they can be seen extensively in other diseases of the peripheral motor neuron as poliomyelitis, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy [15]. Regarding the mechanism of the genesis of fasciculations, it is now known that the fasciculations can occur at any level of a peripheral motor neuron, and the complex fasciculations are particularly frequent in the region of distal axon arborisation [16]. There is an observation in patients with ALS that the fasciculations more often occur in an early phase of the disease when the muscular strength is relatively preserved. However, eliminating carefully other diseases and by the electrophysiological examination, the importance is given to fasciculations in early diagnosis of ALS, so de Carvalho and Swash [17] fasciculation as “very early marker of ALS”.

This research showed that already after six months of monitoring using the AS criteria, more EMG affected regions can be detected, which is consistent with the research of Schrooten et al. [18] who showed that about half of the patients (46.4%) have for at least one more affected region by using the AS criteria in relation to the patients categorised in accordance with the El Escorial criteria.

Having in mind that there are no strict protocols of electrophysiological evaluation related to the number of muscle examinations, or their selection by additional analysis we tried to provide commentary on the significance of this issue. In our sample, fibrillations and PSW are particularly often registered in mBB and mADM in upper extremities in only 2 examinations in a row, where the ADM showed somewhat more frequent presence of fasciculations. Contrary to this, the analysis of the examination of lower extremity muscles showed the particular importance of the small muscles of the feet (mEDB and mFHB) in detecting fasciculations.

In summary, prospectively analyzing the use of new AS criteria for the diagnosis of amyotrophic lateral sclerosis, the preservation of specificity is confirmed, with the additional increase in the sensitivity of this approach. A study like this does not represent a special affirmation of electrophysiological evaluation, having in mind that the approach is unavoidable was derived from the Lambert criteria. However, the AS criteria affirm a different importance of electrophysiological indicators enabling a faster determination of the categories of definite diagnosis and at the same time better accessibility to innovative forms of treatment of this acute fatal disease for the patients.

Conclusion

Application of the AS criteria improve the achievement of a category of probable or definite diagnosis of ALS by 2.7 months earlier compared to the rEE. This outcome is particularly affected by a higher frequency of positive EMG findings, when the AS criteria were employed. Early determination of diagnosis provides the better perspective and more frequent participation of ALS patients in the pharmacotherapy studies intended to establish new therapeutic options.

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Prevalence of metabolic syndrome and the association with sociodemographic characteristics in adult population of Banja Luka

Prevalencija metaboličkog sindroma i njegova povezanost sa sociodemografskim karakteristikama odraslog stanovništva Banje Luke

Verica Petrović*†, Gordana Tešanović*†, Ljiljana Stanivuk†‡

***Health Center Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; University of Banja Luka, †Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; ‡Health Care Institute of Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Metabolic syndrome (MS) is a cluster of metabolic and hemodynamic disorders that increase the risk of developing atherosclerotic cardiovascular diseases and type 2 diabetes mellitus. The aim of this study was to determine the prevalence of MS and its components in adult population of Banja Luka and association with sociodemographic characteristics. **Methods**. A total of 685 participants (348 men and 337 women), aged 18 years and over, were analyzed. The diagnosis of the MS was based on definition set by the International Diabetes Federation (IDF). **Results**. The prevalence of the MS was high (37.5%), slightly higher in women (38.3%) than in men (36.8%), but without statistically significant difference (p = 0.686). Prevalence of each individual component of the MS in the study group was over 30% (systolic blood pressure ≥ 130 mmHg – 42.0%; diastolic blood pressure ≥ 85 mmHg – 31.0%; triglycerides ≥ 1.7 mmol/L – 36.1%; high density lipoprotein (HDL) cholesterol < 1.03 for men and <1.29 for women – 31.2%; glucose ≥ 5.6 mmol/L – 32.8%; central obesity ≥ 94 cm male and ≥ 80 cm female – 62.6%). The prevalence of the MS was not associated with gender, but with age. A number of participants increased with incresed age in the group with the MS with statistically significant difference compared to the group without the MS. The study showed an association between level of education and the MS. Low level of education was associated with the appearance of the MS with statistically significant differences (df = 3; p = 0.013). Association between level of education and the MS was shown in women (df = 3; p = 0.000), but not in men (df = 3; p = 0.883). Retirees and housewives were significantly present in the group with the MS, students and unemployed in the group without the MS, while employed participants showed no statistically significant difference. **Conclusion**: The MS was diagnosed in over one-third of adults in Banja Luka. Prevalence of MS was not associated with gender, but it was associated with age, level of education as well as with some categories of employment.

Key words:
metabolic syndrome; prevalence; socioeconomic factors; demography; age factors; education.

Apstrakt

Uvod/Cilj. Metabolički sindrom (MS) je skup metaboličkih i hemodinamskih poremećaja koji povećavaju rizik od aterosklerotskih kardiovaskularnih bolesti i dijabetes melitusa tipa 2. Cilj rada bio je da se utvrdi prevalencija MS i njegovih komponenti kod odraslog stanovništva Banja Luke, kao i povezanost MS sa sociodemografskim karakteristikama. **Metode.** Analizirano je ukupno 685 ispitanika (348 muškaraca i 337 žena), starosti 18 godina i više. MS je procenjivan na osnovu definicije Internacionalnog udruženja za dijabetes (*International Diabetes Federation* – IDF). **Rezultati**. Prevalencija MS bila je visoka (37,5%), nešto viša kod žena nego kod muškaraca, ali bez statistički značajne razlike (38,3% : 36,8%; p = 0.686). Zastupljenost svake pojedinačne komponente MS iznosila je preko 30% (sistolni krvni pritisak ≥ 130 mmHg – 42,0%; dijastolni krvni pritisak ≥ 85 mmHg – 31,0%; trigliceridi ≥ 1,7 mmol/L – 36,1%; lipoproteini velike gustine HDL holesterol < 1,03 muškarci i < 1,29 žene – 31,2%; glikemija ≥ 5,6 mmol/L – 32,8%; centralna gojaznost ≥ 94 cm muškarci i ≥ 80 cm žene – 62,6%). Pokazalo se da pojava MS u ispitivanoj grupi nije zavisila od pola, ali jeste od godina života. Sa povećanjem godina života zastupljenost ispitanika se povećavala u grupi sa MS (statistički značajna razlika u odnosu na grupu bez MS). Potvrđena je poveza-

Correspondence to: Verica Petrović, Health Center Banja Luka, Zdrave Korde 4, 78 000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina. E-mail: drverica@hotmail.com

nost pojave MS sa stepenom obrazovanja. Nizak nivo obrazovanja bio je udružen sa pojavom MS [statistički značajna razlika (df = 3; p = 0.013)]. Udruženost između stepena obrazovanja i MS je potvđena kod žena (df = 3; p = 0.000), ali ne i kod muškaraca (df = 3; p = 0.883). Penzioneri i domaćice bili su statistički značajno zastupljeniji u grupi sa MS, a studenti i nezaposleni u grupi bez MS, dok kod zaposlenih nije bilo statistički značajne razlike. **Zaključak**. MS je zastupljen kod više od jedne trećine odraslog stanovništva Banja Luke. Pokazalo se da pojava MS nije zavisila od pola, ali je postojala zavisnost u odnosu na životno doba, stepen obrazovanja, kao i određenih kategorija radnog statusa.

Ključne reči:
metabolički sindrom; prevalenca; socioekonomski faktori; demografija; životno doba, faktori; obrazovanje.

Introduction

Metabolic syndrome (MS) is a collection of cardiometabolic abnormalities that represent a risk for type 2 diabetes, coronary heart disease and other cardiovascular diseases [1]. The World Health Organization (WHO) in 1998 proposed a definition of the MS on the basis of previous studies [2]. WHO definition put diabetes or impaired fasting glycaemia or impaired glucose tolerance or insulin resistance as a mandatory criterion for the MS. Besides, a person must have 2 more following risk factors: diagnosed hypertension (≥ 140/90 mmHg), dyslipidemia (elevated triglycerides ≥ 1.7 mmol/L or low level of high density cholesterol (HDL) cholesterol – for male < 0.9 mmol/L and for female < 1.0 mmol/L, obesity determined by body mass index ≥ 30 kg/m² or waist/hip ratio > 0.9 (male) or > 0.85 (female) and microalbuminuria (≥ 20 μg/min). It was pointed out that this was only a framework of definitions and that there was the need to work on its improvement.

Today there are more definitions used in research which makes it difficult to compare results. There are definitions in use which do not set any component as a condition for assessing the presence of the MS. These are the Third Report of the National Cholesterol Education Program (NCEP) Expect Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panal III – ATP III) and that of the National Heart, Lung, and Blood Institute (NHLBI). According to these definitions, presence of 3 components represent the MS, but using different limits for individual components. For example, according to the ATP III definition [3] the MS exists when 3 or more of the following factors are fulfilled: 1. central obesity: waist circumference > 102 cm (male) or > 88 cm (female); 2. triglyceridemia (≥ 1.7 mmol/L); 3. low HDL cholesterol: < 1.0 mmol/L (male) and < 1.3 (female); 4. blood pressure ≥ 135/85 mmHg or medication; 5. fasting plasma glucose ≥ 6.1 mmol/L.

One of recent definition is the definition of the International Diabetes Federation (IDF) [4]. IDF definition made abdominal obesity a mandatory criterion with cut-points for waist circumference based on gender and ethnicity, for Europian men ≥ 94 cm and women ≥ 80 cm. According to the IDF definition, diagnosis of the MS is based on the presence of abdominal obesity plus any 2 out of 4 risk factors.

Several associations (IDF Task Force on Epidemiology and Prevention, NHLBI, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity) continued to debate criteria for diagnosis of the MS and in 2009 a harmonized definition was released [Joint Interim Statement (JIS) definition]. JIS definition excludes abdominal obesity as a mandatory risk factor while making the presence of any 3 out of 5 risk factors (waist circumference ≥ 94 cm – male and ≥ 80 cm – female; triglyceridemia ≥ 1.7 mmol/L or medication; low HDL cholesterol < 1.0 mmol/L – male and < 1.3 – female or medication; blood pressure ≥ 130/85 mmHg or medication; fasting plasma glucose ≥ 5.6 mmol/L or diabetes mellitus diagnosis) neccessary for diagnosis of the MS [5].

In addition to the parameters that are included in the definitions as the MS risk factors, the influence of lifestyle and sociodemographic factors on the occurrence of the MS should be investigated.

The aim of this study was to determine the prevalence of the individual components of the MS as well as association with socio-demographic factors in adult population of Banja Luka.

Methods

A population-based cross-sectional survey was conducted in 2012 on adult population of Banja Luka, registered in the Family Medicine at the Health Center. According to the Report of the Health Care Fund of the Republic of Srpska, at the time of the study, the Health Center had 175,000 registered citizens. The target group in this study was 18 years of age and over. There were 142,000 registered citizens of the appropriate age. In relation to that number, a representative sample of 700 respondents was formed.

The survey instrument was the questionnaire containing: personal data of the patient, age, gender, education, employment status, the results of 3 consecutive measurements of blood pressure, waist size, and laboratory analyses (fasting glycemia and lipids).

All participants were devided into 4 groups according to their level of education (no schooling, incomplete primary or primary education, secondary school, post-secondary and university education).

The interviews were conducted by 103 family medicine teams. The second and third measurements of blood pressure were averaged and this value was used for further analysis. For each patient, the presence of the MS was determined by using the IDF definition. According to this definition, a person has the MS if, in addition to abdominal obesity, has 2 more of the following risk factors: blood pressure ≥ 130/85

mmHg or already diagnosed hypertension; triglycerides ≥ 1.7 mmol/L; HDL cholesterol < 1.03 mmol/L for men (M) and < 1.29 mmol/L for women (F) or previously treated this lipid disorders; morning fasting glycemia ≥ 5.6 mmol/L or already diagnosed diabetes [4]. At the end of the study, we obtained 685 properly filled out questionnaires from which the data was entered into the database in the SPSS computer program. The response rate was 97.85%.

Data analysis was done using descriptive statistical methods. The significance of differences between groups, or different categories of respondents, was calculated by using the Pearson's χ^2 test. The results are presented in tables.

The research was conducted in accordance with the Declaration of Helsinki as revised in 2013. Before entering the research each participant gave written informed consent.

Results

This study included 685 participants aged 18 years and more, 348 (50.8%) men and 337 (49.2%) women. The representation of women and men was approximately the same (Table 1). The mean age of respondents was 48.77 ± 17.888 years, for women 50.40 ± 18.964 and men 47.20 ± 16.657. The mean age of subjects with the MS (55.87 ± 15.762) was higher than of those without the MS (44.51 ± 17.751). Most of the respondents were employed – 285 (41.7%), and the second most represented were retired persons – 180 (26.4%). By level of education most of them had secondary school – 392 (58.1%) (Table 1).

Table 1

The prevalence of metabolic syndrome (MS) by sociodemographic characteristics in the population of Banja Luka

Characteristics	MS, n (%)			
	total 685 (100)	yes 257 (37.5)	no 428 (62.5)	*p**
Gender				
males	348 (50.8)	128 (49.8)	220 (51.4)	df = 1
females	337 (49.3)	129 (50.2)	208 (48.6)	0.686
Age (years)				
18 – 29	125 (18.0)	18 (7.0)	105 (24.5)	
30 – 39	134 (19.6)	34 (13.2)	100 (23.4)	
40 – 49	97 (14.2)	30 (11.7)	67 (15.7)	df = 5
50 – 59	132 (19.3)	61 (23.7)	71 (16.6)	0.000
60 – 69	100 (14.6)	64 (24.9)	36 (8.4)	
70+	99 (14.5)	50 (19.5)	49 (11.4)	
Employment status				
employed	285 (41.7)	98 (38.1)	187 (43.9)	
self-employed	18 (2.6)	7 (2.7)	11 (2.6)	
retiree	180 (26.4)	103 (40.1)	77 (18.1)	df = 6
housewives	46 (6.7)	26 (10.1)	20 (4.7)	0.000
students	54 (7.9)	1 (0.4)	53 (12.4)	
unemployed	98 (14.3)	22 (8.6)	76 (17.8)	
unable to work	2 (0.3)	0 (0.0)	2 (0.5)	
Level of education				
total				
no schooling	27 (4.0)	15 (5.9)	12 (2.8)	
incomplete primary or elementary education	91 (13.5)	44 (17.1)	47 (11.1)	df = 3
secondary school	392 (58.1)	140 (55.3)	252 (59.7)	0.013
post-secondary and university education	165 (24.4)	54 (21.3)	111 (26.3)	
males				
no schooling	3 (0.9)	1 (0.8)	2 (0.9)	
incomplete primary or elementary education	32 (9.3)	11 (8.7)	21 (9.7)	df = 3
secondary school	212 (61.6)	76 (59.8)	136 (62.7)	0.883
post-secondary and university education	97 (28.2)	39 (30.7)	58 (26.7)	
females				
no schooling	24 (7.3)	14 (11.1)	10 (4.9)	
incomplete primary or elementary education	59 (17.8)	33 (26.2)	26 (12.7)	df = 3
secondary school	180 (54.4)	64 (50.8)	116 (56.6)	0.000
post-secondary and university education	68 (20.5)	15 (11.9)	53 (25.9)	

***Pearson's χ^2 test**

Table 2

The prevalence of metabolic sindrome (MS) components in the population of Banja Luka

Parameters	MS, n (%)			
	total 685 (100)	yes 257 (37.5)	no 428 (62.5)	*p**
Elevated SBP (≥ 130 mmHg)	292 (42.7)	166 (64.8)	126 (29.4)	0.000
Elevated DBP (≥ 85 mmHg)	212 (31.0)	129 (50.4)	83 (19.4)	0.000
Triglycerides (≥ 1.7 mmol/L)	246 (36.1)	161 (62.9)	85 (20.0)	0.000
HDL cholesterol (< 1.03 M; < 1.29 F)	209 (31.2)	127 (50.0)	82 (19.7)	0.000
Glucose (≥ 5.6 mmol/L)	223 (32.8)	167 (65.5)	56 (13.2)	0.000
Central obesity (≥ 94 cm M; ≥ 80 cm F)	429 (62.6)	257 (100.0)	172 (40.2)	0.000

***Pearson's χ^2 test; SBP systolic blood pressure; DBP – diastolic blood pressure; HDL – high density lipoprotein; M – male; F – female.**

The prevalence of the MS in the population registered at the Health Center Banja Luka, according to the IDF definition, was 37.5%. The prevalence was slightly higher in women than in men, but without statistically significant difference (Table 1).

It has been shown that the occurrence of the MS in the study group did not correlate with gender, but did correlate with age. With increasing age, the occurrence of the MS was higher in the group of patients with the MS with a statistically significant difference compared to the group without the MS (Table 1). In the group of patients with the MS, prevalence increased with age and reached a maximum in the group of 60–69 years (24.9%), followed by the group aged 50–59 years (23.7%).

By employment status, in the study group, the largest group were the employed patients (41.7%). There were no statistically significant differences in the representation of the employed and self-employed in the groups with and without the MS (Table 1). As a special form of employment status, retirees and housewives were singled out and they were significantly over-represented in the group with the MS compared to the group without the MS, while students and unemployed were significantly more frequent in the group without the MS.

Our study showed association between level of education and the MS (Table 1). The low level of education was associated with the appearance of the MS with statistically significant differences (df = 3; $p = 0.013$). The association between level of education and the MS was shown in women (df = 3; $p = 0.000$), but not in men (df = 3; $p = 0.883$). Women with out schooling and incomplete or finished primary education were significantly over-represented in the group with the MS, and women with post-secondary and university education in the group without the MS.

The risk factors included in the IDF definition (Table 2) were highly represented in the total study group, each with more than 30%. The most common risk factors were central obesity (62.6%) and systolic blood pressure (42.7%), accompanied by other risk factors that were more frequently statistically significant in the group with the MS compared to the group without the MS.

Discussion

The prevalence of the MS in the adult citizens of Banja Luka registered at the Health Center is high. More than one-third of the adult population has the MS that is in accordance with recently published study in the Republic of Srpska [6]. Research conducted in Croatia, in a family medicine setting, showed even higher prevalance of 45% [7]. According to a meta-analysis by Kastorini et al. [8], the MS occurrence in the world according to the IDF definition ranges from about 7.4% to about 50%. In Western countries, the prevalence of the MS according to the IDF definition is lower comparing to the results of our research, ranging from 20% to 30% (for example in Spain, 24.3%) [9].

The varying definitions make some study comparisons more difficult. By a certain definition of the NCEP ATP III, the MS prevalence in the US population older than 20 years was 33% during the period from 2003 to 2012 [10]. The same definition of the MS used in the Iranian urban population showed a very high prevalence of 42.3% [11].

There was no statistically significant difference in the MS prevalence between women and men in our study. Results of studies around the world related to the prevalence of the MS by gender are different. Al-Daghri et al. [11] showed that women in Saudi Arabia had a significantly greater prevalence of the MS than men (47.2% to 40.3%, respectively) according to the ATP III definition,. The mentioned American study [10] showed that the prevalence of the MS is significantly higher in women than in men (35.6% v.s. 30.3%, $p < 0.001$). Another US study evaluating the occurrence of the MS of 5,227 adult African-Americans, also showed greater occurrence of the MS in women (40%) than in men (27%), according to the NCEP ATP III [12].

There is a positive association between the MS and age. Representation of patients in the group with the MS increased with age from 7.0% in the group aged 18 to 29 years to 24.9% in the group of 60 to 69 years old, and over the age 70 there was a slight decrease in prevalence. Brazilian LATINMETS study [13] also confirmed the association between the MS prevalence and age, especially over the age of 40 years. Almost all researchers in the world who have addressed this issue confirmed the occurrence of the MS in association with age [1, 14–16].

It turned out that among the employed participants, there were no statistically significant difference in the occurrence of the MS, while the unemployed were significantly less represented in the group with the MS (8.6% to 17.8%; $p = 0.001$; $p < 0.05$). Research conducted in Saudi Arabia showed a statistically significant lower incidence of the MS among the unemployed in comparison to all other work categories [11]. For our respondents, we singled out two groups – retirees and housewives who had a high presence of the MS. Both are highly statistically more frequent in the group with the MS.

Our study confirmed the association between the MS prevalence and the level of education. Respondents without education and with incomplete primary and finished primary education were significantly more common in the group with the MS than in the group without the MS, which is in accordance with most studies in the world. Research on the adult Saudi population was accompanied by the emergence of the MS through 3 levels of education: school up to 6 years, 7–12 years of school and more than 12 years of school, and the significantly lower incidence of the MS was confirmed in those who had more than 12 years of education compared to the other two groups[11]. A recent study conducted in China including 15,477 urban population adult subjects age 18–74 years showed that higher education is associated with a higher prevalence of the MS in men, but lower among women[16] which is in accordance with our results. In the present study, men with post-secondary and university education were more frequently represented in the group with the MS than in the group without the MS but without statistically significant difference, while women with post-secondary and university education were represented statistically significantly lower in the group with the MS. Khan et al. [12] showed that the low level of education in African-American women was associated with the emergence of the MS, but not in African-American men.

Prevalence of each individual component of the MS in our study group was over 30%. Among the factors contributing to the MS, the most prevalent was central obesity (62.6%), followed by elevated systolic blood pressure (42.7%), elevated triglycerides (36.1%), elevated blood glucose (32.8%) and low HDL (31.2%). Our results are similar to the results of the study from the North-East China that reported similar results [16]. The results of the great Europiean project MORGAN, investigating the MS in 10 European countries, showed that the individual factors of the MS were dependent upon the age of the subjects. The most prevalent factor in young women was obesity, in older women blood pressure, whereas in men of all ages elevated blood pressure was the dominating factor [1].

Conclusion

The prevalence of the MS was detected in over one-third of the adult residents in Banja Luka, while prevalence of each individual component of the MS in the study group was over 30%. The most common risk factors were central obesity followed by blood pressure. The prevalence of the MS was not associated with gender but with age.

The association between level of education and the MS was confirmed in women, but not in men.

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Quality of life in patients with chronic liver disease

Kvalitet života bolesnika sa hroničnom bolešću jetre

Dušan Dj. Popović*†, Darija Kisić Tepavčević†‡, Nada Kovačević*†, Tamara Milovanović*†, Miodrag Krstić*†, Ivan Ranković*, Jelena Martinov*†, Tijana Glišić*†, Rada Ješić*†, Tatjana Pekmezović†‡

Clinical Centre of Serbia, *Clinic for Gastroenterology and Hepatology, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, ‡Institute of Epidemiology, Belgrade, Serbia

Abstract

Background/Aim. Quality of life is impaired in patients with the chronic liver disease (CLD). Patients with this disease have numerous disabling problems which lead to a reduced health related quality of life (HRQoL). The aim of our study was to evaluate the predictive value of selected socio-demographic and clinical characteristics on HRQoL in Serbian cohort of patients with the CLD. **Methods.** Over a period of one year, we performed a study which included patients with the CLD. We used Short Form Health Survay-36 (SF-36) for assessment of HRQoL. The assessment of depression and anxiety was made by using Hamilton scale of depression and anxiety, while the assessment of fatigue was performed by Fatigue severity scale. **Results.** The study included 103 patients with the CLD. The average values of the overall SF-36 scores were 52.6 ± 20.4, while the mean score of the composite scores were 53.5 ± 19.6 for the Mental component summary and 49.8 ± 21.3 for the Physical component summary. Some domains of HRQoL were significantly affected by following factors: gender, age, employment status, alcohol consumption, depression, anxiety and fatigue. Predictors of physical components of HRQoL were employment, depression and fatigue, and predictors of mental components were depression and fatigue. **Conclusion.** The tested socio-demographic, clinical and behavioral factors have an impact on the HRQoL in patients with the CLD. The most important predictors of HRQoL are behavioral factors suggesting the need for an adequate therapeutic action in order to improve the HRQoL in these patients.

Key words:
liver diseases; hepatic insufficiency; quality of life; serbia; sociological factors; demography; depression; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Kvalitet života je snižen kod bolesnika sa hroničnom bolešću jetre (HBJ). Bolesnici sa ovom bolešću imaju veliki broj onesposobljujućih simptoma što dovodi do redukcije kvaliteta života povezanog sa zdravljem (KŽPZ). Cilj ovog istraživanja bio je evaluacija prediktivne vrednosti sociodemografskih i kliničkih karakteristika na KŽPZ u srpskoj kohorti bolesnika sa HBJ. **Metode.** Sprovedena je studija preseka u trajanju od jedne godine, koja je uključivala bolesnike sa HBJ. Za procenu KŽPZ korišćen je *Short Form Health Survay-36* upitnik (SF-36). Za procenu depresije i anksioznosti korišćene su Hamiltonova skala depresije i anksioznosti, dok je procena zamora vršena skalom težine zamora. **Rezultati.** U studiju je bilo uključeno 103 bolesnika sa HBJ. Prosečna vrednost ukupnog SF-36 skora je bila 52,6 ± 20,4. Vrednosti kompozitnih skorova bili su 53,5 ± 19,6 za mentalni kompozitni skor i 49,8 ± 21,3 za fizički kompozitni skor. Na pojedine domene kvaliteta života utiču: pol, starost, zaposlenje, konzumiranje alkohola, depresija, anksioznost i zamor. Prediktori fizičke komponente kvaliteta života su bili: zaposlenje, depresija i zamor, dok su prediktori mentalne komponente bili depresija i zamor. **Zaključak.** Od ispitivanih sociodemografskih, kliničkih i bihejvioralnih faktora samo pojedini imaju uticaja na KŽPZ bolesnika sa HBJ. Najvažniji prediktori kvaliteta života su bihejvioralni faktori, što ukazuje na potrebu adekvatnog terapijskog delovanja u cilju poboljšanja KŽPZ kod ovih bolesnika.

Ključne reči:
jetra, bolesti; jetra, insuficijencija; kvalitet života; srbija; socijalni faktori; demografija; depresija; ankete i upitnici.

Correspondence to: Dušan Dj. Popović, Clinical Centre of Serbia, Clinic for Gastroenterology and Hepatology, Dr Koste Todorovića 6, Belgrade 11 000, Serbia. E-mail: pduschan@gmail.com

Introduction

Over the past decades it has been highlighted that physical aspect of health is predominant from physician's viewpoint, while patients emphasize the importance of how they feel and how disease affects their well-being [1]. Integration of the biomedical model of health with socio-medical model (psychosocial and economic components) resulted in the appearance of a new concept – health-related quality of life (HRQoL) [2]. This concept is related to a patient's subjective assessment of physical, mental and social dimensions of well-being and social functioning [3].

HRQoL assessment is carried out using standardized questionnaires which may be generic or specific. The most widely used generic questionnaire is SF-36 (Short Form Health Survay-36) [4]. The various disease-specific questionnaires are available in the field of Hepatology, but most commonly used is CLDQ (Chronic Liver Disease Questionnaire) [5].

It has been well recognized that patients with chronic liver disease (CLD) have deeply affected HRQoL [6–12]. Namely, patients with this disease have numerous problems (depression, anxiety, loss of self-esteem, emotional problems, fatigue, itching, complications of the liver cirrhosis, reduced working capacity, etc.) leading to reduced HRQoL and well-being [1, 2, 13–16].

Although the numerous and heterogeneous studies were conducted in order to find out the influence of the CLD on HRQoL, the majority of these investigations were based on the importance of etiology [1, 6, 14, 15, 17–23] and severity of the CLD [1, 2, 6, 14-16, 19, 20–22, 24–26] on HRQoL. The most often evaluated predictors of HRQoL among cohort of the CLD patients were: gender [6, 12, 14–16, 19–23, 27], age [6, 14, 16, 19–23, 27, 28], ascites [15, 23], hepatic encephalopathy [8, 23], depression and anxiety [16, 18, 21, 26, 28–30] and fatigue [12, 18, 26–28]. Furthermore, the possible influence of level of education, employment and marital status on HRQoL is available from studies conducted on patients with liver transplantation [31] and patients with the CLD using the disease specific questionnaire [16, 19]. On the other hand, there is a shortage of information about the importance of factors such as children status, alcohol consumption, smoking, duration of liver disease and previous gastrointestinal bleeding on the HRQoL of patients with the CLD.

Keeping in mind all mentioned above, the aim of our study was to evaluate the predictive value of selected sociodemographic and clinical characteristics, on HRQoL in Serbian cohort of patients with the CLD.

Methods

Patients

The sampling method and detailed methodology were published previously [30, 32]. We performed a cross-sectional study for a period of one year (October 2009 – October 2010). The study was conducted at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, and included 103 patients with chronic liver disease (chronic hepatitis or cirrhosis). Exclusion criteria were: age < 18 years, psychiatric disorders, acute complications of the CLD, hepatic encephalopathy (grade > 2) and liver transplantation [30, 32].

Instruments

The SF-36 was used as a general questionnaire [4]. It consisted of 36 questions, grouped into eight domains: Physical functioning (PF), Role physical (RP), Bodily pain (BP), General health (GH), Vitality (VT), Social functioning (SF), Role emotional (RE) and Mental health (MH). In addition to calculating these scores, two composite scores were calculated. Physical component summary (PCS) included domains: Physical functioning, Role physical, Bodily pain and General health, while Mental component summary (MCS) included Vitality, Social functioning, Role emotional and Mental health. The total SF-36 score represented mean value of the PCS and MCS. Higher values denoted better HRQoL.

Behavioral factors

The severities of depression and anxiety were measured using the Hamilton depression scale (HDRS) [33] and Hamilton anxiety scale (HARS) [34]. The Hamilton depression scale included evaluation of 21 symptoms or signs of depression which are graded on a scale ranging from 0 (best score) to 4 (worst score), or from 0 to 2 (for some items). The Hamilton anxiety scale consisted of 14 questions that assessed the level of anxiety (semi-quantitative), where the answers were ranked from 0 (best score) to 4 (worst score). In both Hamilton scales the total score is equal to the sum of individual scores, and higher score indicated higher degree of depression or anxiety. The grade of fatigue was determined by the Fatigue severity scale (FSS) [35]. The FSS scale evaluated 9 claims on the scale from 1 ("strongly disagree") to 7 ("strongly agree"). A total score was equal to the sum of scores for each statement divided by 9. Higher total score indicated higher level of fatigue.

Ethics

The study was approved by the Ethics Committee of the Faculty of Medicine, University in Belgrade (No. 29/I-2).

Statistics

We used methods of descriptive and analytical statistics. Testing the significance of differences was performed by Student's *t*-test or one-way ANOVA (parametric variable), and χ^2 test, Mann-Whitney U test or Kruskal-Wallis H test (nonparametric variable). In case of statistical significance, post hoc Tukey tests or multiple Kruskal-Wallis tests were used. We used Pearson's or Spearman's correlation coefficients for analyzing correlation. The significant difference was set for $p < 0.05$.

An effect of individual variables on the composite scores (PCS and MCS) was assessed by univariate and hierarchical multivariate regression analysis.

Results

The study was included 103 patients with chronic liver disease, 56 male and 47 females, with average age of 53.8 ± 12.9 years. The largest proportion of patients (71.8%) were unemployed. Most of the patients had the CLD in a stage of liver cirrhosis (77%), usually of alcoholic etiology (35%). The average SF-36 scores were 52.6 ± 20.4. The average MCS was 53.5 ± 19.6, while PCS was 49.8 ± 21.3.

Gender

In comparison to men, the women had significantly lower MCS [t (101) = 2.149; p = 0.034], PCS [t (101) = 2.132; p = 0.035] and scores for the domains: Physical functioning (z = -2.483; p = 0.013) and Mental health [t (101) = 2.459; p = 0.016], as presented in Table 1. For the domains: Role physical (p = 0.148), Bodily pain (p = 0.212), Vitality (p = 0.067), General health (p = 0.549), Social functioning (p = 0.053) and Role emotional (p = 0.238) no significant differences were found.

Age

A statistically significant difference was detect for PCS [F (2.100) = 4.852; p = 0.010] and the scores for the domains Physical functioning [χ^2 (2) = 17.275; p < 0.001] and Bodily pain [χ^2 (2) = 7.359; p = 0.025] (Table 1). Post hoc analysis showed that patients aged ≤ 39 years had higher PCS and sub-score for Physical functioning and Bodily pain compared to the patients ≥ 60 years. The patients aged 40–59 years had a significantly higher sub-score for Physical functioning and Bodily pain compared to the patients aged ≥ 60 years. In the domains Role physical (p = 0.270), General health (p = 0.648), Vitality (p = 0.077), Social functioning (p = 0.410), Role emotional (p = 0.159), Mental health (p = 0.828) and MCS (p = 0.445) no significant differences were found.

Level of education

Among the patients with different education levels, no difference was found in MCS (p = 0.814), PCS (p = 0.580) as well as in scores for the domains Physical functioning (p = 0.944), Role physical (p = 0.387), Bodily pain (p = 0.135), General health (p = 0.799), Vitality (p = 0.617), Social functioning (p = 0.613), Role emotional (p = 0.715) and Mental health (p = 0.498).

Employment status

The patients with different employment status had a statistically significant difference in scores for the domains Physical functioning [χ^2 (2) = 18.857; p < 0.001], Role physical [χ^2 (2) = 6.145; p = 0.046], Bodily pain [χ^2 (2) = 15.763; p = 0.001], Vitality [F (2,100) = 5.857; p = 0.004] and PCS [F (2.100) = 9.377; p < 0.001] (Table 1).

Post hoc analysis showed that patients who were retired had a significantly lower score in the domains Physical functioning, Role physical, Bodily pain, Vitality, and PCS

Table 1

The values of SF-36 scores between the groups with significant difference in at least one domen

	SF-36 score*									
Characteristics	Physical functioning	Role physical	Bodily pain	General Health	Vitality	Social functioning	Role emotion	Mental health	MCS	PCS
Gender										
male	**67.1 (28.7)**	37.5 (43.4)	64.0 (28.4)	41.5 (19.5)	59.1 (22.4)	67.4 (26.9)	47.0 (42.5)	**71.5 (19.0)**	**57.3 (19.9)**	**53.8 (22.0)**
female	**53.5 (28.5)**	24.4 (37.4)	56.7 (28.8)	39.3 (16.1)	50.9 (22.2)	56.3 (30.1)	36.8 (42.4)	**61.8 (20.6)**	**49.0 (18.6)**	**45.0 (19.6)**
Age (years)										
≤ 39	**81.0 (19.3)**	46.6 (46.1)	**72.8 (28.9)**	40.0 (19.2)	64.6 (19.6)	70.0 (30.9)	53.3 (39.4)	69.0 (24.2)	59.4 (21.7)	**61.0 (18.6)**
40–59	**65.3 (28.0)**	30.7 (39.7)	**63.8 (26.6)**	42.1 (18.2)	56.7 (21.8)	59.1 (26.4)	34.6 (41.7)	67.6 (18.2)	52.0 (19.2)	51.7 (20.5)
≥ 60	**46.1 (27.8)**	26.3 (40.5)	**51.0 (29.3)**	38.4 (17.5)	49.5 (23.7)	63.8 (31.3)	49.0 (43.9)	65.5 (21.7)	53.3 (19.5)	**42.3 (21.2)**
Employment										
employed	**74.3 (25.5)**	**44.8 (46.4)**	**75.3 (26.3)**	42.2 (20.4)	**64.3 (20.6)**	63.3 (26.0)	48.2 (47.6)	69.1 (19.9)	57.4 (21.4)	**60.2 (20.1)**
unemployed	**70.2 (29.6)**	**36.0 (40.8)**	**65.6 (26.8)**	41.8 (17.4)	**59.6 (18.8)**	64.0 (30.4)	44.0 (38.1)	70.0 (18.0)	55.9 (17.6)	**54.6 (20.3)**
retired	**48.2 (26.2)**	**21.4 (35.7)**	**49.5 (26.8)**	38.8 (17.0)	**47.9 (23.3)**	60.9 (30.0)	38.0 (41.9)	64.4 (21.5)	50.0 (19.4)	**41.2 (19.3)**
Consuming alcohol										
no	**54.4 (28.9)**	22.3 (35.8)	59.7 (30.4)	38.2 (19.0)	53.5 (23.3)	59.8 (31.2)	39.7 (42.0)	63.6 (21.0)	51.0 (19.9)	45.6 (20.4)
yes	**66.3 (28.7)**	39.2 (43.9)	61.4 (27.4)	42.4 (17.1)	56.9 (22.0)	64.5 (26.7)	44.6 (43.2)	70.0 (19.3)	55.7 (19.4)	53.3 (21.6)

***Mean (± standard deviations); SF-36 – Short Form Health Survay-36; PCS – Physical component summary; MCS – Mental component summary; Bold – p < 0.05.**

compared to the employed patients and in the domains Physical functioning, Bodily pain, and PCS compared to the unemployed patients. In the domains of General health (p = 0.676), Social functioning (p = 0.893), Mental health (p = 0.434), Role emotional (p = 0.620) and MCS (p = 0.221) no significant differences were found.

Marital status

Patients with different marital status did not differ in scores regarding the domains MCS (p = 0.618), PCS (p = 0.677), Physical functioning (p = 0.501), Role physical (p = 0.794), Bodily pain (p = 0.707), General health (p = 0.807), Vitality (p = 0.631), Social functioning (p = 0.953), Role emotional (p = 0.918) and Mental health (p = 0.063).

Children

Among patients who had and those who did not have children there were no statistically significant differences in MCS (p = 0.289) and PCS (p = 0.926) as well as scores in the domains Physical functioning (p = 0.983), Role physical (p = 0.855), Bodily pain (p = 0.598), General health (p = 0.728), Vitality (p = 0.276), Social functioning (p = 0.984), Role emotional (p = 0.192) and Mental health (p = 0.448).

Alcohol consumption

Patients who consumed alcohol had significantly higher scores in the domain Physical functioning (z = -2.124; p = 0.034) (Table 1). As for the domains Bodily pain (p = 0.759), Role physical (p = 0.055), General health (p = 0.251), Vitality (p = 0.439), Social functioning (p = 0.416), Role emotional (p = 0.582), Mental health (p = 0.114), PCS (p = 0.071) and MCS (p = 0.229) no significant differences were found.

Smoking

Among smoking and nonsmoking patients, we found no statistically significant differences in MCS (p = 0.428), PCS (p = 0.889), Physical functioning (p = 0.986), Role physical (p = 0.884), Bodily pain (p = 0.725), General health (p = 0.503), Vitality (p = 0.504), Social functioning (p = 0.208), Role emotional (p = 0.495) and Mental health (p = 0.830).

Disease severity

Among the patients with varying severity of the CLD no significant difference was found neither in PCS (p = 0.742) and MCS (p = 0.883), nor in scores for the domains Physical functioning (p = 0.764), Role physical (p = 0.418), Bodily pain (p = 0.355), General health (p = 0.979), Vitality (p = 0.565), Social functioning (p = 0.553), Role emotional (p = 0.256) and Mental health (p = 0.318).

Etiology

Among patients with different etiology of the CLD no significant differences were found in PCS (p = 0.608), MCS (p = 0.283) nor in scores for the domains Physical functioning (p = 0.181), Role physical (p = 0.844), Bodily pain (p = 0.728), General health (p = 0.766), Vitality (p = 0.541), Social functioning (p = 0.120), Role emotional (p = 0.408) and Mental health (p = 0.303).

Duration of the chronic liver disease

There was no significant correlation between the duration of liver disease and MCS (p = 0.078), PCS (p = 0.700), Physical functioning (p = 0.958), Role physical (p = 0.159), Bodily pain (p = 0.372), General health (p = 0.153), Vitality (p = 0.439), Social functioning (p = 0.303), Role emotional (p = 0.112) and Mental health (p = 0.058).

Previous gastrointestinal bleeding

Among patients who had episode of gastrointestinal bleeding and those who did not, no significant differences in MCS (p = 0.319), PCS (p = 0.699), Physical functioning (p = 0.965), Role physical (p = 0.060), Bodily pain (p = 0.589), General health (p = 0.687), Vitality (p = 0.562), Social functioning (p = 0.660), Role emotional (p = 0.167) and Mental health (p = 0.500) were found.

Ascites

Among patients with and those without ascites, there were no statistically significant differences in MCS (p = 0.536), PCS (p = 0.392), and in scores for the domains Physical functioning (p = 0.987), Role physical (p = 0.785), Bodily pain (p = 0.655), General health (p = 0.831), Vitality (p = 0.256), Social functioning (p = 0.905), Role emotional (p = 0.078) and Mental health (p = 0.239).

Depression and anxiety

HDRS score was significantly correlated with the both composite scores and all sub- scores of the SF-36. The highest correlation was with MCS, while the lowest one was with Social functioning (Table 2).

Anxiety

HARS score was significantly correlated with both composite scores and all sub-scores of the SF-36. The highest correlation was found with Vitality and MCS, while the lowest one was with Bodily pain (Table 2).

Fatigue

FSS score was significantly negatively correlated with both composite scores and all sub-scores of the SF-36. The highest correlation was found with PCS, while the lowest one was with Social functioning (Table 3).

Table 2

Correlation coefficients between the SF-36 and HARS, HDRS and FSS

	SF-36 scale									
Scale	Physical functioning	Role physical	Bodily pain	General Health	Vitality	Social functioning	Role emotion	Mental health	PCS	MCS
HDRS	-0.573*	-0.467*	-0.392*	-0.457*	-0.618*	-0.339*	-0.501*	-0.593*	-0.610*	-0.655*
HARS	-0.479*	-0.348*	-0.289*	-0.425*	-0.622*	-0.304*	-0.406*	-0.551*	-0.542*	-0.601*
FSS	-0.670*	-0.504*	-0.394*	-0.420*	-0.653*	-0.338*	-0.457*	-0.339*	-0.681*	-0.593*

SF-36 – Short Form Health Survay-36; PCS – Physical component summary; MCS – Mental component summary; HARS – Hamilton Anxiety Rating Scale; HDRS – Hamilton Depression Rating Scale; FSS – Fatigue severity scale.
***Correlation is significant at the 0.01 level.**

Table 3

Hierarchical regression analysis of the Physical component summary

Variable	Model I			Model II			Model III		
	B	SE(B)	β	B	SE(B)	β	B	SE(B)	β
Gender	-5.03	4.05	-0.12	-3.22	3.44	-0.08	-1.83	2.91	-0.04
Age	-0.08	0.19	-0.05	-0.02	0.16	-0.14	0.03	0.14	0.02
Employment									
retired	Reference group			Reference group			Reference group		
employed	16.07	5.68	0.34**	11.85	4.81	0.25*	8.64	4.09	0.18*
unemployed	10.90	5.90	0.22	7.20	4.94	0.15	6.31	4.17	0.13
HARS				0.24	0.46	0.07	0.44	0.39	0.13
HDRS				-1.36	0.31	0.59**	-1.02	0.27	-0.45**
FSS							-4.82	0.77	-0.47**
R^2		0.167**			0.434**			0.601**	
F for change in R^2		4.866**			22.337**			39.511**	

HARS – Hamilton Anxiety Rating Scale; HDRS – Hamilton Depression Rating Scale; FSS – Fatigue severity scale; *$p < 0.05$; ** $p < 0.01$.

Predictors of HRQoL

Hierarchical regression analysis showed that socio-demographic variables (gender, age, employment) explained 16.7% of the variance ($p < 0.01$) of PCS as outcome measure. Addition of the variables "depression and anxiety" in the second model caused an increase of 26.7% in the variance explanation ($p < 0.01$). Furthermore, after adding the "fatigue" in the third block, an additional 16.7% of the variance in PCS was explained ($p < 0.01$). The final model described that gender, age, employment, HDRS, HARS and FSS accounted for 60.1% of the variance in PCS. The results in the final block have shown that employment ($p < 0.05$), depression ($p < 0.01$) and fatigue ($p < 0.01$) significantly influenced physical dimension of HRQoL (Table 3).

With MCS as dependent variable, the first model, consisting of selected socio-demographic variables, accounted for 6.0% of the variance in the outcome variable. Moreover, depression and anxiety explained additional 39.1% in the total change in MCS in this analysis ($p < 0.01$). Fatigue, in the third model, accounted an additional 10.7% of the variance in MCS ($p < 0.05$). The final model explained 55.8% of the variance in MCS ($p < 0.01$) (Table 4). Among all investigated variables statistically significant impact on the mental component of quality of life was observed only for depression and fatigue (Table 4).

Table 4

Hierarchical regression analysis of the Mental component summary

Variable	Model I			Model II			Model III		
	B	SE(B)	β	B	SE(B)	β	B	SE(B)	β
Gender	-6.28	3.98	-0.11	-4.23	3.14	0.12	-3.20	2.48	-0.08
Age	0.12	0.19	0.08	0.19	0.15	0.12	0.22	0.13	0.14
Employment									
retired	Reference group			Reference group			Reference group		
employed	7.34	5.58	0.17	2.07	4.38	0.05	-0.27	3.98	-0.01
unemployed	6.49	5.79	0.14	2.32	4.50	0.05	-0.27	3.98	-0.06
HARS				-0.16	0.42	0.05	-0.02	0.38	0.01
HDRS				-1.28	0.29	-0.61**	-1.04	0.26	-0.49*
FSS							-3.56	0.75	-0.38**
R^2		0.06			0.451**			0.558**	
F for change in R^2		1.556			33.844**			22.700**	

HARS – Hamilton Anxiety rating scale; HDRS – Hamilton Depression Rating Scale; FSS – Fatigue severity scale; *$p < 0.05$; ** $p < 0.01$.

Discussion

Over the past decade, the various studies have identifed that patients with the CLD had deteriorated HRQoL [1, 8, 12]. In the sample of 103 patients, we tried to explain comprehensively, HRQoL in patients with the CLD. The SF-36 scores were significantly different in patients with different gender, age, employment status and alcohol consumption.

In our study, women had significantly lower HRQoL than men in the domains of MCS, PCS, Physical functioning and Mental health. These results correspond to literature data [12, 14, 20, 27]. The study of Afendy et al. [20] described that women with the CLD had significantly lower scores for the majority SF-36 domains. In patients with cholestatic liver disease, women had significantly poorer scores for the Physical functioning domain [14], whereas women with chronic hepatitis C had significantly worse Physical functioning, Role physical, PCS and MCS [21, 27].

With regard to age, we found that younger patients had significantly better scores for the Physical functioning, Bodily pain and PCS, compared to the older patients, as was expected. Our results are similar to those of Younossi et al. [14], who described that patients younger than 50 years, had higher scores in the domains PCS, Physical functioning and Role physical, compared to the older ones. In the study of Afendy et al. [20], association between age and all scales of SF-36 was found. However, the literature also provides evidence that age has no impact on HRQoL in this patient group [1, 6, 12, 16, 21, 22].

Furthermore, the patients with different employment status had a statistically significant different score for the PCS and several SF-36 domains. These scores were worst in the retired patients. Positive impact of employment on the patient's wellbeing was a reason for all higher scores among the employed patients. The retired patients had affected physical domains of HRQoL, predominantly, and the reason for this may be the fact that the retired elderly patients often suffer from additional comorbidity. This was supported by the fact that the retirees had poorer HRQoL for the same domain (except for Role physical and Vitality) than unemployed patients who were on average younger.

By performing the hierarchical multiple regression analysis, we came to a conclusion that employment was a significant predictor of physical components of HRQoL, but not mental ones. The study of Kim et al. [16] found that the employed patients with liver cirrhosis had better HRQoL than the unemployed and that employment was a significant predictor of HRQoL. The authors explained that disease most commonly affected men in their most productive period of life, and that their employment status was directly associated with their role function at home or at work.

The patients who consumed alcohol had a significantly better score for the Physical functioning domain. The reason for this result was not known.

Previously published studies described that the marital status [16, 19] and level of education [16], did not affect HRQoL in patients with the CLD. Our study confirmed these results. However, Saab et al. [31] described that patients who underwent liver transplantation, married patients as well as patients who had more than 12 years of education had significantly higher scores for the Physical functioning domain, while there was no difference regarding other scores.

The main difference between our and previously published studies is the impact of the severity of the CLD on HRQoL. The literature contains data that patients with severe disease have worse HRQoL, as measured by SF-36 [2, 10, 20, 23, 25] or CLDQ [6, 14, 19, 22, 24]. According to the SF-36 domains, Sobhonnslidsuk et al. [25] described the significant decline in all domains, Younossi et al. [2] all domains except Vitality, while Les et al. [23] described the significant decline in all domains except General health and Mental health. In our study, we obtained results that indicate that the severity of the disease does not significantly affect any of the SF-36 questionnaire scores. A clear gradient of the reduction in HRQoL score was only registered for the domains Physical functioning and Bodily pain, and perhaps for them the difference would be significant if the sample was higher. It was registered for almost all physical components of HRQoL domains that patients with noncirrhotic or early cirrhotic (Child-Pough A) CLD had higher scores compared to the patients with advanced cirrhosis (Child-Pough C). This regularity did not follow the scores of mental component of HRQoL. Specifically, in our study, scores for MCS and Mental health domains were highest in the patients with advanced cirrhosis, which was previously described [1, 2, 17]. The reason for that was that the clinical progression of the CLD predominantly affects the physical dimension of HRQoL with spared mental dimension [2, 14]. The literature has described that in patients with the CLD-hepatocellular type the increased severity of disease did not follow the deterioration of Bodily pain, Vitality, Role emotional, Mental health and MCS domains [14]. In our cohort, 56.4% were patients with hepatocellular liver disease (alcoholic etiology and other), while only 15.5% of patients had the viral CLD. In the study of Afendy et al. [20], Sobhonslidsuk et al. [25], and Les et al. [23] predominantly viral etiology (42% to 65 %) was presented. Given that our sample was most similar to a sample of Younossi et al. [2], it is possible that it is because of social and cultural differences in our and other cohorts. In addition, in our country, the support program for the patients with the CLD often has no access to modern treatment for this disease group, while liver transplants are under development. This has a negative effect on HRQoL, regardless of the clinical stage of the disease. In our cohort, Child Pough score had no predictive value for PCS and MCS, which was described by Les et al. [23] and Häuser et al. [21]. In our model, the socio-demographic and behavioral factors had predictive value.

The results of our study indicate that patients with different CLD etiology did not differ in any SF-36 score of the questionnaire, which is consistent with previously published studies [1, 6, 15–17, 21].

Depression is a common disorder in patients with the CLD, especially those with chronic hepatitis C [18, 36, 37]. About 60% of patients with the CLD have depression [38]. It is known that the presence of depression may lead to deterioration of physical condition and functioning of a patient [36]. In patients

awaiting for liver transplantation, those who suffer from depression have higher mortality rate than those without depression [37]. The presence of the CLD, knowledge of its stigmata and outcome and social effects of the disease can lead to depression. Also, many patients with the CLD come from population that is vulnerable to appearance of psychiatric disorders. Depression is a negative predictor of HRQoL of patients with the CLD [10, 16, 21, 26], as confirmed by our research. In our predictive model, depression and anxiety were significant predictors of physical and mental components of HRQoL.

Fatigue is a common symptom in patients with the CLD, which has a significant impact on their HRQoL [39]. It is particularly pronounced in some types of the CLD (cholestatic liver disease and hepatitis C) [12, 40]. Analyzed by domains, fatigue affect the most the domains General health, Social functioning and Mental health [12]. Our study confirmed that fatigue is a significant predictor of physical and mental components of HRQoL, which is consistent with previously published results [12, 26, 29, 39, 40]. Since the exact mechanism of fatigue in the CLD is unknown, specific therapy is not available [39]. Because of the significant correlation between fatigue and depression appearing in our and other studies [36], treatment of depression might have an indirect influence on improving HRQoL by reducing fatigue.

Conclusion

The tested sociodemographic, clinical and behavioral factors have an impact on HRQoL in patients with the CLD. The most important predictors of HRQoL are behavioral factors which suggest the need for an adequate therapeutic action in order to improve the HRQoL in these patients.

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The sociodemographic characteristics and risk factors for tuberculosis morbidity between two decades at the beginning of the 21st century at the north of Kosovo, Serbia

Sociodemografske karakteristike i faktori rizika od oboljevanja od tuberkuloze između dve dekade na početku 21. veka na severu Kosova, Srbija

Sonja Smiljić*, Dejana Stanisavljević†, Blagica Radović‡, Milica Mijović§, Sladjana Savić§, Siniša Ristić‖, Predrag Mandić§

University of Priština/Kosovska Mitrovica, Faculty of Medicine, *Department of Physiology, §Department of Pathology, Kosovska Mitrovica, Serbia; University of Belgrade, Faculty of Medicine, †Institute for Medical Statistics and Informatics, Belgrade, Serbia; Clinical-Health Center, ‡Department of Pulomonology, Kosovska Mitrovica, Serbia; University of East Sarajevo, Faculty of Medicine, ‖Department of Physiology, Foča, Bosnia and Herzegovina

Abstract

Background/Aim. Tuberculosis (TB) is a major cause of mortality and morbidity worldwide, affecting different countries disproportionally. Effective diagnosis and treatment of TB saved an estimated 43 million lives between 2000 and 2014. The aim of our study was to show sociodemographic influences, risk factors for the morbidity and clinical characteristics of tuberculosis among residents of northern Kosovo, Serbia, between two decades at the beginning of the 21st century. **Methods.** A prospective study was conducted at the Department for Pulmonology of the Clinical-health Centre, Kosovska Mitrovica, included all tuberculosis patients treated during two three-year periods, between 2000–2002 and 2012–2014. In total, 134 patients with tuberculosis were treated, 91 in the first observed period and 43 in the second period. **Results.** In both observed periods, male patients suffered from tuberculosis more frequently. In the first observed period, younger and middle age (18–49) persons suffered from tuberculosis more frequently ($p = 0.014$). In relation to the place of residence, in the first reporting period there were more patients from urban areas, while in the second, there were significantly more patients from rural areas ($p = 0.008$). In the second observed period, TB was significantly more associated with chronic obstructive pulmonary disease ($p = 0.001$) and comorbidities with TB were significantly more frequent ($p = 0.006$). During the 2000–2002 period, there were more severe clinical forms, with severe radiological changes, bilateral parenchymal and cavernous forms ($p = 0.08$). Mild unilateral parenchymal lesions were more common in the last 3 years ($p = 0.02$). **Conclusion.** Social determinants, older age and comorbidities are the most important risk factors for the persistent number of patients, and therefore this target group needs attention during active approach in TB screening.

Key words:
tuberculosis; sociological factors; demography; risk factors; morbidity.

Apstrakt

Uvod/Cilj. Tuberkuloza (TB) je vodeći uzrok mortaliteta i morbiditeta širom sveta zahvatajući nesrazmerno razne zemlje. Tačna i proverena dijagnoza i adekvatno lečenje prema procenama sačuvali su 43 miliona života na početku 21. veka. Cilj studije bio je da se ukaže na sociodemogtafski uticaj, faktore rizika za oboljevanje i kliničke karakteristike tuberkuloze kod stanovnika severnog Kosova, Srbija, između dve dekade na početku 21. veka. **Metode.** Prospektivnom studijom obuhvaćene su obolele osobe od tuberkuloze lečene na Grudnom odeljenju Kliničko-bolničkog centra u Kosovskoj Mitrovici, tokom dva trogodišnja perioda, između 2000–2002. i 2012–2014. godine. Lečena su ukupno 134 bolesnika obolela od tuberkuloze, 91 u prvom periodu i 43 u drugom posmatranom periodu. **Rezultati.** U oba perioda bilo je više obolelih muškaraca. U prvom posmatranom periodu, osobe mlade i srednje životne dobi (18–49) značajno češće su oboljevale od tuberkuloze ($p = 0.014$). U odnosu na mesto stanovanja, u prvom posmatranom periodu bilo je više obolelih koji žive u gradu, dok je u drugom periodu, značajno više bolesnika bilo sa

Correspondence to: Sonja Smiljić, University of Priština, Faculty of Medicine, Department of Physiology, Anri Dinana bb, Kosovska Mitrovica, Serbia. Email: sonja.smiljic@med.pr.ac.rs

sela ($p = 0.008$). U drugom posmatranom periodu, TB je bila značajno povezana sa hroničnom obstruktivnom bolešću pluća ($p = 0.001$) i komorbiditeti su bili značajno češći ($p = 0.006$). Tokom perioda 2000–2002. godina, bile su zastupljenije teže kliničke forme, sa ozbiljnim radiološkim promenama, bilateralane parenhimatozne i kavernozne forme ($p = 0.08$). Umerene unilateralne parenhimatozne lezije su bile češće u drugom trogodišnjem periodu ($p = 0.02$).

Zaključak. Socijalne determinante, starija životna dob i komorbiditeti su najvažniji faktori rizika za održavanje perzistentnog broja obolelih i zato je to ciljna grupa na koju treba obratiti posebnu pažnju u skriningu tuberkuloze.

Ključne reči:
tuberkuloza; socijalni faktori; demografija; faktori rizika; morbiditet.

Introduction

Tuberculosis (TB) is a major cause of mortality and morbidity worldwide, affecting different countries disproportionally. Effective diagnosis and treatment of TB saved an estimated 43 million lives between 2000 and 2014 [1].

According to the World Health Organization (WHO) and the European Centre for Disease Prevention and Control, a decrease in the frequency of tuberculosis has been registered [2]. The Republic of Serbia registered less than 20 cases per 100,000 inhabitants which represents a low incidence rate of tuberculosis [3]. At the same time, it is reported that Kosovo has a higher incidence of tuberculosis diseases compared to other areas of the Balkans. Compared with the other neighbouring areas, such as Albania or Macedonia, the incidence of tuberculosis in Kosovo is twice as high [4, 5].

The decreasing trend in number of new cases of tuberculosis is accompanied by the change of sociodemographic and clinical characteristics of the disease. In the middle of the last century, the number of younger TB patients was significantly higher while the contact with the infected persons was a common reason for the occurrence of the disease [6, 7]. Fibrocaseous and cavernous phthisis were manifested as more severe clinical manifestations, which required long-term treatment and were often accompanied by the disease relapse with approximately 30% [8–10]. At the end of the 1990's, pulmonary tuberculosis kept characteristics of a social disease with a morbidity peak in the fourth decade of life and clinical features dominated by the parenchymatous form with cavities and rare, but persistent, miliary pulmonary tuberculosis (1.9% to 2.4%) [11]. These characteristics of the tuberculosis has remained as such up to date, but only in regions with a high incidence of disease, mainly in Africa and Asia [11, 12].

In the last decade in areas with a lower incidence of TB, it often affects elderly, over 40% [13–15], while in countries with a high rate of disease the most vulnerable are people in their middle age [12, 16]. The clinical TB features are often disguised by symptoms of comorbidities such as diabetes mellitus (DM) and chronic obstructive pulmonary disease (COPD) thus making the diagnosis identification more difficult [17]. Characteristic of the old age is that the tuberculous changes may occur in the lower lung fields, which is atypical for pulmonary tuberculosis (PTB) [9, 13, 18].

In the last two decades, social determinants and risk factors changed. Social and economic reasons (place of residence, occupation) as well as habits (smoking, alcohol consumption and drug use) remained equally important [19]. In contemporary society, the improvement of living standard increased the number of patients suffering from cardiovascular diseases, diabetes or obstructive lung disease. The comorbidity with diabetes mellitus is inviolable. DM is documented in all groups of patients with TB, but the association of the DM and TB is significantly higher in the elderly and in the obese – ones with greater waist circumference. Comorbidities are risk factors that contribute to the incidence of diseases such as TB that is now more common in the elderly [9, 17]. Tuberculosis is a major health problem and it is a difficult task for doctors to suspect tuberculosis and recognize the typical symptoms of the disease without proper consideration of social determinants and risk factors. One of the reasons for continual maintenance of new cases of tuberculosis in most areas of the world is the increasing number of human immunodeficiency virus (HIV) – positive patients [1, 2].

There is insufficient published data on the number of newly discovered cases of tuberculosis as well as risk factors for the maintenance of the number of patients in population in the northern Kosovo, Serbia. The aim of our study was to examine sociodemographic influences, risk factors for the morbidity and clinical characteristics of tuberculosis among residents of the northern Kosovo by analysis and comparison of the two three-year periods, 2000–2002 and 2012–2014.

Methods

The survey was conducted in full respect of the ethical principles and was approved by the Ethics Committee of the Faculty of Medicine, University of Priština with temporary seat in Kosovska Mitrovica.

A prospective study was conducted at the Department of Pulomonology of the Clinical-health Centre in Kosovska Mitrovica, the reference institution for the tuberculosis treatment. The study included patients with tuberculosis who had been treated in the period between 2000 and 2002 and in the period between 2012 and 2014. All hospitalized patients were divided into 2 groups, depending on the observed period of time and compared afterwards.

On admission, the patients' data regarding demography, age, gender, body weight as well as the initial symptoms of the disease were processed. Patients were considered to have positive symptoms of cough, expectoration, weight loss, night sweats and fatigue if these symptoms had been present for two or more weeks. Haemoptysis were positive if it occurred only once.

The following social determinants have been processed: place of residence, family status, education and occupation. Also, we processed risk factors of getting infected with tu-

berculosis, including previous tuberculosis and anti-tuberculosis treatment, the possibility of contact with persons suffering from tuberculosis, alcohol consumption, smoking, drug use, prolonged use of corticosteroids, the use of immunosuppressive therapy and comorbidities such as diabetes mellitus, chronic renal failure, cancer, chronic obstructive pulmonary disease, liver cirrhosis, congestive heart failure and HIV infection.

The results of the lung affection on chest radiography were categorized according to the extent of changes, their localization and approximately to their morphological structure. Presence or absence of cavity was the basis of TB division to initial and advanced forms confirmed by radiologist. Sputum samples were taken from all the patients for direct microscopy of the preparations coloured according to Ziehl-Neelsen method. Also, a cultivation of bacillus on Lowenstein-Jansen medium was performed for all samples. Sputum was collected in the morning, before eating, after a spontaneous expectoration. Each sputum positive for direct microscopy was verified by the culture on Lowenstein-Jansen medium. Pulmonary tuberculosis was bacteriologically confirmed if the two sputum findings confirmed bacillus and/or in the case of positive sputum cultivation. Chest radiographies have been also performed. Each abnormality in the lungs was confirmed by the radiologists.

Descriptive statistics were calculated for the sociodemographic characteristics, risk factors, and clinical characteristics of patients with tuberculosis who had been treated in two observed periods. Differences between groups were analyzed by using Pearson's χ^2 test for categorical variables. All tests were two-tailed. $p < 0.05$ was considered statistically significant. The IBM SPSS 21(Chicago, IL, 2012) package was used for these analyses.

Results

The study included all tuberculosis patients treated during two three-year periods, between 2000 and 2002 and between 2012 and 2014. In total, 134 patients with tuberculosis were treated, 91 in the first observed period and 43 in the second one. The differences in sociodemographic characteristics in two periods, from the very beginning of the 21th century and 10 years after, were examined. In both observed periods, male patients suffered from tuberculosis more frequently (Table 1).

In the first observed period, younger and middle aged (18–49) people suffered from tuberculosis more frequently. Among the patients, treated between 2012 and 2014, there were significantly more persons older than 50 years of age ($p = 0.014$). In relation to the place of residence, in the first reporting period there were more patients from urban areas, while in the second, significantly more patients were from rural areas ($p = 0.008$). Social determinants, such as family and employment status as well as education level did not differ between the observed periods and represent a variable that has remained unchanged in the observed period as well as important factor determining morbidity of tuberculosis (Table 1).

Table 1

Sociodemographic characteristics of patients with tuberculosis (TB) (n= 134)

Variables	2000–2002 n (%)	2012–2014 n (%)	*p* value
Numbers of patients	91	43	
Sex			
male	60 (65.9)	29 (67.4)	
female	31 (32.1)	14 (32.6)	0.512
Age group, years			
18–29	15 (16.5)	7 (16.3)	
30–39	18 (19.8)	5 (11.6)	
40–49	23 (25.3)	5 (11.6)	
50–59	12 (13.2)	14 (32.6)	
≥ 60	23 (25.3)	12 (27.9)	< 0.014*
Residence			
urban	49 (46.2)	10 (76.7)	
rural	42 (53.8)	33 (23.3)	< 0.001
Marital status			
married	49 (53.8)	19 (45.2)	
single	42 (46.2)	24 (55.8)	0.195
Employment status			
employed	55 (60.4)	31 (72)	
unemployed	33 (39.6)	12 (28)	0.098
Education			
no education	5 (5.5)	3 (7)	
primary	22 (22.2)	5 (11.6)	
secondary	61 (67)	32 (74.4)	
More than secondary	3 (3.3)	3 (7)	0.289

Table 2

Comparison of the risk factors in patients with active tuberculosis (TB) between 2000–2002 and 2012–2014 (n = 134)

Variables	2000–2002 n (%)	2012–2014 n (%)	*p* value
Social determinant	33 (36.3)	12 (26.6)	0.225
TB contact	16 (17.6)	6 (14)	0.398
History of anti-TB treatment	15 (16.5)	4 (9.3)	0.201
Smoking status	41 (45.1)	22 (51.2)	0.317
Alcohol status	22 (24.2)	14 (32.6)	0.207
Daibetes mellitus	3 (3.3)	5 (11.6)	0.070
COPD*	3 (3.3)	10 (23.3)	0.001
Comorbidity	28 (30.8)	21 (49)	0.006

***COPD – chronic obstructive pulmonary disease.**

Table 3

Comparison of clinical characteristics of patients with tuberculosis (TB) between two periods, 2000–2002 and 2012–2014 (n = 134)

Variables	2000–2002 n (%)	2012–2014 n (%)	*p* value
Site of TB			
pulmonary	83 (91.2)	37 (86)	
extrapulmonary	8 (8.8)	6 (14)	0.292
Treatment category			
new	82 (90)	39 (90.7)	
retreatment	9 (10)	4 (9.3)	0.484
Clinicals characteristics			
Parenchymal involvement			
unilateral	6 (21.4)	11 (61.1)	
bilatral	22 (78.6)	7 (38.9)	0.008
cavities	55 (60.4)	18 (41.9)	0.005
Radiological severity			
initial	6 (7.2)	11 (30.6)	
advanced TB	77 (92.8)	25 (69.4)	0.002
Mycobacteriology characteristics of total TB /sputum or smear			
negative	36 (39.6)	15 (34.9)	
positive	55 (60.4)	28 (65.1)	0.373

Socioeconomic factors, smoking and alcohol consumption are the risk factors for developing TB, but there was no distinction between the observed periods. In the second observed period, with a greater number of respondents older than 50 years, TB was significantly more associated with chronic obstructive pulmonary disease ($p = 0.001$) and comorbidities which were significantly more frequent ($p = 0.006$) (Table 2). One of the most important risk factors, HIV infection, could not be listed, since there were not HIV positive among the patients.

The occurrence of pulmonary tuberculosis was more frequent than extrapulmonary (Table 3). Similarly, the number of newly found cases was higher than relapse ones and there was no significant difference between groups. There was significant difference in clinical manifestations of the disease 15 years ago and now. At the very beginning of the century, there were more severe clinical forms, with severe radiological changes, bilateral parenchymal and cavernous forms ($p = 0.08$). Mild unilateral parenchymal lesions were more common over the last 3 years ($p = 0.02$). There were no significant differences Mycobacterial characteristics and confirmation of the disease, did not differ significantly in the examined periody (Table 3).

Discussion

This is the first study on social determinants and risk factors for developing tuberculosis at the north of Kosovo. At the same time, it delivers clinical and radiographic features in patients with tuberculosis in two observed periods.The study provides original descriptive data on the profile of the TB patients, social determiners and risk factors of morbidity in northern Kosovo during the two 3-years periods with the time distance of 10 years. The trend of tuberculosis morbidity rate in the Republic of Serbia declined in last 10 years. In 2012, the rate of incidence in Serbia was 23/100,000, while in Kosovo, it was 47/100,000 [1]. The high rate of incidence in Kosovo could be the result of numerous factors including the low level of health protection of the local population, poverty and low level of education [4, 5].

As a result of good organization and effective operation of anti-tuberculosis dispensaries in Serbia, a low incidence of AIDS and rare resistant strains of *Mycobacterium tuberculo-*

sis, the incidence and mortality are significantly reduced, despite the worsening of socioeconomic conditions during the 1990's [14]. According to the data of the World Health Organization, the largest number of tuberculosis patients in Serbia in 2014 were older than 65 years and predominantly males. Age and sex were observed elsewhere as strong determinants of TB disease with a higher risk of TB disease observed among older individuals and men. The distribution of patients, according to urban/rural place of residence varied between the groups. According to 15 years old data, patients from the urban area were predominant, but in the last 3 years patients from rural areas prevail. The migrations of population after 2000 in Kosovo and Metohija changed the structure of the population at the north of Kosovo between the two observed periods. The data in the literature differ; some state that there are more patients in urban areas [20], and some show that patients from rural areas prevail [16, 21].

Family status, as a social determinant, was not different in the observed periods. Employment status is an important social determinant. There is no significant difference between the compared groups, but in the period 2000–2002, there were more patients without employment. During that period, among TB patients, there were more displaced persons with the undefined employment status. Although there were significantly more patients with TB among those with primary and secondary education [16], there was no significant difference in the observed period.

In addition to socioeconomic status as a factor for tuberculosis morbidity, contacts with patients and previous history of tuberculosis treatment represent important risk factors. Increased duration of exposure is associated with the risk of tuberculosis infection [22].

Tobacco smoking is associated with significantly increased risks of latent tuberculous infection, active tuberculosis, TB recurrence and mortality [23]. These results are consistent with other studies in which tobacco smoke was found to alter mucociliary function and the clearance of inhaled substances, promote the adherence of bacteria to the epithelial cells of the airways, increase alveolar permeability and reduce humoral and cell-mediated immunity. This would facilitate *Mycobacterium tuberculosis* infection, while also subsequently favouring the onset of disease and its increased severity [24]. Approximately half of our respondents, in both observed periods, were smokers, with a long-time habit of smoking. The link between smoking and tuberculosis was described in many studies [12, 20, 25, 26]. The habit of tobacco smoking is, even nowadays, still represented in the population of northern Kosovo, and therefore aggressive campaigns about the harmfulness of tobacco might lead to the reduction in the number of tuberculosis patients [27].

Low to moderate alcohol intake is not associated with the tuberculosis morbidity. On the other hand, persons who drink more than 40 g/day or have alcohol use disorders are at significant risk. Persons who regularly consume alcohol suffer more frequently from severe clinical forms and have poorer treatment outcomes [12, 26, 28].

Alcohol can have direct toxic effects on the immune system. Chronic alcohol consumption directly reduces macrophage activity and cellular immunity and inhibits activity of the tumor necrosis factor [29]. Increased risk for developing tuberculosis among alcohol users can be explained by the pattern of behaviour- staying in bars, shelters, prisons and social institutions [30, 31].

WHO reports indicate that DM is associated with a significantly increased risk of TB morbidity, treatment failure and disease relapse or fatal outcome [32]. The comorbidity of diabetes mellitus and tuberculosis has been confirmed in several studies, regardless of the study design or geographical area in which they are conducted [26, 33, 34]. High incidence of DM and pre-DM in adults suffering from TB was noted, which results in more severe clinical features and poorer tuberculosis treatment outcomes. Diabetes is associated with multi-drug resistant tuberculosis (MDR-TB) in patients without a previous history of tuberculosis. In patients with diabetes, cellular immunity is suppressed, which is a key defence mechanism against *Mycobacterium tuberculosis*. Macrophages and T-lymphocytes have the most important role in the protection of the organism against tuberculosis. Therefore, attention should be paid to the treatment of latent tuberculosis in diabetics.

The global burden of diabetes mellitus (DM) is immense, with numbers expected to rise to over 550 million by 2030. TB is an infectious disease of equally great antiquity as DM, with evidence of spinal involvement being found in Egyptian mummies dating back to several thousand years BC. There is now strong evidence that there is an important association between DM and TB and that this association results in poor TB treatment outcomes. Heightened clinical suspicion for TB is needed for people with DM, especially among those who live in TB-endemic areas, and systematic screening should be considered [35]. The results of our study indicate a necessity of expansion of public health programs that connect TB and DM, diagnostic and therapeutic measures.

Comorbidities, especially chronic obstructive pulmonary disease, represent a significant risk factor for developing tuberculosis and poorer treatment outcome. In the period between 2012 and 2014, in which the majority of respondents were older than 50 years of age, there were significantly more comorbidities. WHO ranked COPD as a significant risk factor for tuberculosis [19]. An increasing number of people are suffering from COPD, and it may be due to habit of tobacco smoking and life in urban areas with serious air pollution. The significance of comorbidities for developing tuberculosis is described in numerous studies [12, 26, 34].

In northern Kosovo, there are significantly more patients with pulmonary tuberculosis. The number of patients suffering from extrapulmonary tuberculosis (EPTB) is lower than in some underdeveloped countries with high incidence of TB, or in countries with more HIV-infected persous [18, 36]. The diagnosis in our respondents was made based on mycobacterial confirmation of the disease, with direct microscopy sputum for bacillus cultivation, which was positive in a significant number of patients, but with no differences for both observed periods [32, 36]. Clinical features and radiographic characteristics differed in the two observed periods. Tuberculosis that affects both lungs ($p = 0.008$) and cavernous form ($p = 0.005$) were significantly more frequent in

the period between 2000 and 2002. In the last 3 years, unilateral parenchymal form was more frequent. Lung radiographic findings indicated that milder changes were recorded in the second study group, while 15 years ago, moderate and extensive radiological changes were observed ($p = 0.002$). In the second observed period, there were more persons aged 50 and older with less extensive radiographic changes, similar to data from the other studies [34].

Unemployment, alcohol consumption and smoking are important social determinants and comorbidities in the elderly are the most significant risk factors for developing TB.

Conclusion

Sociodemographic factors relating to unemployment, lower level of education and poorer housing conditions were not significantly different between the reporting periods. Among treated TB patients in the period between 2000 and 2002 there were more unemployed, working-age young man from urban areas who suffered from severe clinical form of TB with extensive radiographic changes. Comorbidities were rare. The link between smoking and alcohol consumption and the morbidity of tuberculosis is confirmed.

In the period between 2012 and 2014, there were significantly more patients from rural areas older than 50 years. Comorbidities were more frequent, especially DM and COPD.

Immunodeficiency diseases, especially HIV, are not predictors for developing TB in the northern Kosovo nowadays. Social determinants, older age and comorbidities are the most important risk factors for the persistent number of patients, and therefore this target group needs attention during the TB screening.

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Antipsychotics use in the community of Thessaloniki, Greece

Primena antipsihotika na teritoriji Soluna, Grčka

Paraskevi Papaioannidou, Paschalina Kasviki

Aristotle University of Thessaloniki, Faculty of Medicine, Department of Pharmacology, Thessaloniki, Greece

Abstract

Background/Aim. Under the current financial crisis in Greece, an effort has been made by the Greek health authorities to encourage generic prescribing, in order to lower medicinal cost. The purpose of this work was to study antipsychotics use, and to calculate utilization of generics in antipsychotics sales in a sample from the medicines market of Thessaloniki, the second largest city in Greece. **Methods.** A sample of antipsychotics registered sales was collected using the new Electronic Health Records that have been applied in pharmacies during the last years in Greece. The sample corresponded to a small amount of sales from the market of Thessaloniki during the period July 2014–June 2015, including only community and no hospital sales. All brand names (prototype and generics) of antipsychotics and their relative ratios in the sales were estimated, and the percentage of generics in the sale of each medicine was calculated. The amount of medicines was estimated in Defined Daily Doses (DDDs) of the reference/prototype drug and its generics. **Results.** Olanzapine, quetiapine, haloperidol and risperidone sales corresponded to 77% of total antipsychotics sales with percentages of sales 25%, 19%, 19% and 14% respectively. The percentage of sales of other antipsychotics was 7% for amisulpride, 6% for aripiprazole, 4% for ziprasidone and 3% for clozapine. Generic use corresponded to 41% of total sales of antipsychotic drugs (10.884 DDDs out of 26.433 DDDs). Concerning second generation antipsychotics, generic use was high for amisulpride, olanzapine, risperidone and quetiapine. **Conclusion.** In the study sample, second generation antipsychotics corresponded to 78% of sales in the community of Thessaloniki. Haloperidol utilization was also notable. Considering Greek practices, the percentage of generics in antipsychotics sales was very high even for some of the newest antipsychotics.

Key words:
antipsychotic agents; drugs, generic; prescription drugs; economics, pharmaceutical; greece.

Apstrakt

Uvod/Cilj. Tokom ekonomske krize u Grčkoj, zdravstvene vlasti su, u cilju smanjenja troškova u zdravstvenoj delatnosti, stimulisali propisivanje i korišćenje generičkih oblika lekova. Cilj istraživanja je bio analiza potrošnje generičkih lekova 12 grupa antipsihotika na osnovu njihovog prometa u apotekama u Solunu, drugog po veličini grada u Grčkoj. **Metode.** Uzorak prodatih registrovanih antipsihotika formiran je na osnovu elektronskog zdravstvenog kartona, koji je u primeni tokom poslednjih nekoliko godina u apotekama Grčke. Uzorak se odnosio na vanbolničku potrošnju lekova na teritoriji Soluna tokom perioda jul 2014–jun 2015. godine, a ne i na primenu lekova u bolničkim uslovima. Analizirani su originalni i generički oblici antipsihotika koji su bili registrovani u Grčkoj. Izračunata je zastupljenost generičkih oblika lekova u primeni. Obim prometa lekova procenjivan je putem definisane dnevne doze (DDDs) referentnog oblika leka (originalni lekovi i njihovi generični oblici). **Rezultati.** Od ukupnog prometa antipsihotika olanzapin je bio zastupljen sa 25%, kvetiapin sa 19%, haloperidol sa 19% i risperidon sa 14%. Procenat prodaje drugih antipsihotika iznosio je: amisulpirid - 7%, aripiprazol - 6%, ziprasidon - 4% i klozapin - 3%. Upotreba generičkih oblika lekova iznosila je 41% od ukupne prodaje antipsihotika (10,884 od 26,433 DDDs). Kada su u pitanju antipsihotici druge generacije, generički oblici lekova su korišćeni u visokom procentu u slučaju amisulpirida, olanzapina, risperidona i kvetiapina. **Zaključak.** Na ispitivanom uzorku, prodaja antipsihotika druge generacije iznosila je 78% od vanbolničke potorošnje antipsihotika na teritoriji Soluna. Potrošnja haloperidola je bila takođe značajna. Razmatrajući grčku praksu, procenat prodaje generičkih oblika lekova iz grupe antipsihotika je bio veoma veliki, čak i za neke novije antipsihotike.

Ključne reči:
trankvilizeri, veliki; lekovi, generički; lekovi, propisivanje; ekonomija, farmaceutska; grčka.

Correspondence to: Paraskevi Papaioannidou, Aristotle University of Thessaloniki, Faculty of Medicine, Department of Pharmacology, 54124 Thessaloniki, Greece. Phone: +30 2310 999314. E-mail: ppap@auth.gr

Introduction

The use of generics in the Greek medicines market is generally lower than in other European countries [1, 2], accounting for only 11.6% of total pharmaceutical expenditures in 2006. This can be attributed to a general lack of confidence in generics [3, 4], a previous lack of national policy to encourage generic use [5] as well as to small difference in prices between generic and prototype medicines in Greece.

Under the current financial crisis, an effort has been made by the Greek health authorities to encourage generic prescribing in order to lower medicinal cost [6–9].

Published data on generic use in Greece are generally limited and Greece has not participated in large European studies on drug utilization, probably due to, until recently, lack of detailed Electronic Health Records. The purpose of this work was to study antipsychotics use, and to calculate utilization of generics in the community of Thessaloniki – the second largest city in Greece – after the adoption of measures to contain medicinal cost.

Methods

A sample of antipsychotics registered sales was collected using the new Electronic Health Records that have been applied in pharmacies during the last years in Greece [7–9]. The sample corresponded to a small amount of sales from the market of Thessaloniki during the period July 2014 – June 2015, including only community and no hospital sales from the areas of central and Eastern Thessaloniki city and the suburban municipalities of Kalamaria and Panorama. The majority of medicines were obtained after prescribing by a physician, with a negligible percentage (less than 1%) of over the counter sales.

All brand names (prototype and generics) of antipsychotics and their relative ratios in the sales were estimated and the percentage of generics in the sale of each medicine was calculated. The amount of medicines was estimated in daily defined doses (DDDs) of the reference drug and its generics, according to the World Health Organization (WHO) Drug Statistics Methodology, as described in the 2015 Guidelines for Anatomic Therapeutic Chemical (ATC) classification and DDD assignment [9].

Statistical analysis was performed by means of the statistical package SPSS 17.0 (SPSS Inc, Chicago, IL).

Results

In our study sample, all medication products were single-ingredient products. A total of 26,433 DDDs of antipsychotic drugs was sold during the study period in the pharmacies that participated in the study.

Olanzapine, quetiapine, haloperidol and risperidone sales corresponded to 77% of total antipsychotics sales with percentages of sales 25%, 19%, 19% and 14%, respectively. The percentage of sales of other antipsychotics was 7% for amisulpride, 6% for aripiprazole, 4% for ziprasidone and 3% for clozapine. Detailed results on antipsychotics sales can be seen in Table 1 and Figure 1.

Although second generation antipsychotics shared the 78% of the sales in the study sample, haloperidol utilization was also considerable (19%, see Table 1 and Figure 1).

Generic use corresponded to 41% of total sales of antipsychotic drugs (10.884 DDDs out of 26.433 DDDs). The percentage of generics in second generation antipsychotics sales was high for olanzapine (66%), quetiapine (41%), risperidone (59%) and amisulpride (100%). Results on generic use of other antipsycotics can be seen in Table 1 and Figure 1.

The use of prototypes (reference drugs) was high for some of the newest second generation antipsychotics as well as for all first generation antipsychotics that were consumed in our study sample (see Table 1 and Figure 1).

The relative use of reference drugs and generics is presented in Figure 1.

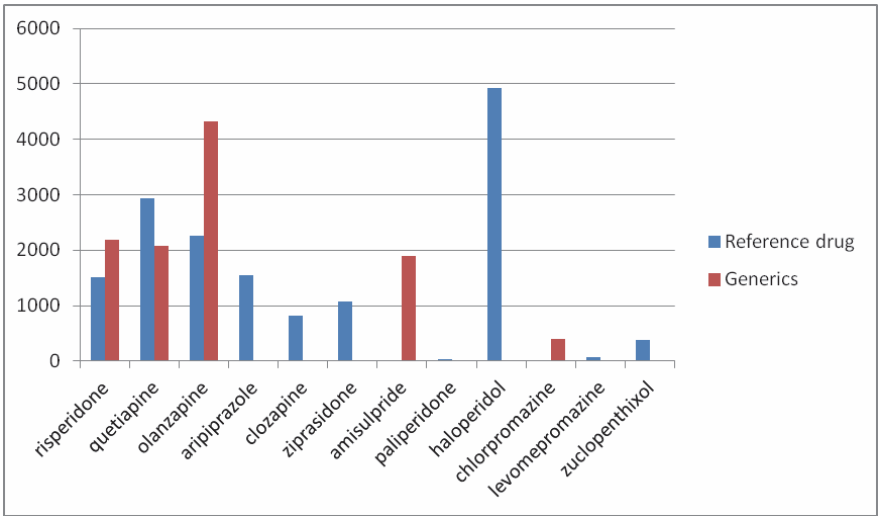

Fig. 1 – Use of generics and reference antipsychotics in the community of Thessaloniki, Greece.

Table 1

Antipsychotics sales in the market of Thessaloniki, Greece

Active Substance		Consumption	
		DDDs	Percentage (%)
Risperidone	Reference drug	1.512	41
	Generic	2.192	59
	Total	3.704	100
Quetiapine	Reference drug	2.936	59
	Generic	2.076	41
	Total	5.012	100
Olanzapine	Reference drug	2.254	34
	Generic	4.326	66
	Total	6.580	100
Aripiprazole	Reference drug	1.549	100
	Generic	0	0
	Total	1.549	100
Clozapine	Reference drug	817	100
	Generic	0	0
	Total	817	100
Ziprasidone	Reference drug	1.078	100
	Generic	0	0
	Total	1.078	100
Amisulpride	Reference drug	0	0
	Generic	1.890	100
	Total	1.890	100
Paliperidone	Reference drug	28	100
	Generic	0	0
	Total	28	100
Haloperidol	Reference drug	4.919	100
	Generic	0	0
	Total	4.919	100
Chlorpromazine	Reference drug	0	0
	Generic	400	100
	Total	400	100
Levomepromazine	Reference drug	69	100
	Generic	0	0
	Total	69	100
Zuclopenthixol	Reference drug	387	100
	Generic	0	0
	Total	387	100
Total	Reference drug	15.549	59
	Generic	10.884	41
	Total	26.433	100

DDDs – Defined Daily Doses.

Discussion

In our study, the most widely used antipsychotics were olanzapine, quetiapine, haloperidol and risperidone (in 77% of cases), followed by amisulpride, aripiprazole, ziprasidone and clozapine. These results are in accordance with the National Institute for Health and Care Excellance (NICE) Guidelines [10] and reflect prescribing patterns similar to those observed in other countries [11–15].

As expected, second generation antipsychotics replaced older antipsychotics and represented the majority of sales in the study (78% of total sales), with olanzapine, quetiapine and risperidone sharing the 58% of sales. Among first generation antipsychotics, haloperidol shared a considerable per-

centage of total use (19%), with minimal utilization of other typical antipsychotics. The high use of haloperidol can be attributed to the efficacy of this medicine in treatment of schizophrenia combined with its low cost. Haloperidol appears to be an attractive cost effective antipsychotic medication, popular in the years of financial crisis in Greece.

The percentage of generics in antipsychotic use was unexpectedly high for Greek standards [2, 8] (41%), being much higher than the percentage of generics in antidepressant use (26%), as observed in a recent study in Greece [7, 16]. This is contrary to the fact that psychiatrists are more likely to choose generic antidepressant than generic antipsychotics [4]. Nevertheless, it is consistent with worsening of depressive symptomatology in cases that antidepressants were switched from original to generics [17]. On the contrary, in the case of antipsychotics, concerns about the interchangeability from original to generics seem to have a realistic basis mainly for clozapine [17]. This is in accordance with our finding that only original clozapine was consumed in our study in Thessaloniki.

The use of generics was generally higher in second generation antipsychotics, with 66% for olanzapine, 59% for risperidone, 41% for quetiapine and 100% for amisulpride. On the contrary, among first generation of antipsychotics, only reference drugs were used. This can be explained by the low price of first generation antipsychotics, which makes the use of generics financially indifferent. On the other hand, the price of second generation antipsychotics is much higher, and this makes generic use financially favorable.

In spite of the general lack of confidence on generic use in antipsychotic therapy [3, 4], the use of generics seems inevitable in order to lower medicinal cost. Nevertheless, in the past there was no difference in price of generics and reference drugs in Greece; there was no motivation for psychiatrists to prescribe generics. In a recent study on utilization of antipsychotics in Greece, the use of generics in 2009 was only 4% [18]. After the financial crisis that hit Greece since early 2010 onwards, a series of measures were implemented to contain public medicines expenditure, including price cuts, changes in reimbursement rates, delisting, internal reference price, international nonproprietary name (INN) prescribing [6, 17]. Although prices of generics were cut by only 10% (before being equal to reference drug prices) [19], the implemented measures encouraged generic prescribing [20]. Due to the high cost of second generation antipsychotics, even this small percentage in the reduction of prices represents a large component of public savings on medicines expenditure. Thus, it seems, that the measures adopted to face the financial crisis really work towards reducing the burden of medical costs.

A limitation of our study is the small sample of sales used. Nevertheless, Thessaloniki is a representative commercial center, and probably the most vivid market, in Greece. In spite of this limitation, our study appears to reflect the real situation in prescribing and consumption of antipsychotics in the community of Thessaloniki [20].

Another limitation of this study is that our sample does not correspond to a specific area or district of the town and the size of population corresponding to consumed antipsychotics in the studied period cannot be estimated; thus, the utilization of antipsychotics cannot be calculated in standard units DDDs/1000 inhabitants/day, and is roughly calculated in DDDs. Nevertheless, our study is one of the few studies on drug utilization in Greece expressing consumption in DDDs and not in cost [18, 20–22] and our results on specific antipsychotics preference seem to be comparable to those observed in other countries [12–15].

Moreover, since there are no major studies on drug utilization in Greece, our study appears to be useful in presenting data on antipsychotics utilization in Greece.

Conclusion

In our study sample, second generation antipsychotics predominated in antipsychotics use in the community of Thessaloniki. Haloperidol utilization was also considerable. The percentage of generics in antipsychotics sales was high (considering Greek practices) even for some of the newest antipsychotics. These results reflect the impact of measures for decreasing medical costs in the years of financial crisis in Greece, and show that psychiatrists try to contain the medical cost within health expenses.

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Mesenchymal stem cells from periapical lesions modulate cytokine production by local immune cells

Mezenhimske matične ćelije iz periapeksnih lezija moduliraju produkciju citokina od strane lokalnih imunskih ćelija

Milan Marković*[†], Sergej Tomić[‡], Jelena Djokić*, Dušan Mihajlović[‡], Dragana Vučević*[‡], Dragan Gazivoda[§], Miloš Duka[§], Miodrag Čolić[‡‖]

Military Medical Academy, *Institute for Medical Research, Clinic for Stomatology, [§]Department for Oral Surgery, Belgrade, Serbia; University of Niš, [†]Faculty of Medicine, Niš, Serbia; University of Defence in Belgrade, [‡]Medical Faculty of the Military Medical Academy, Belgrade, Serbia; [‖]INEP – Institute for Nuclear Energy, University of Belgrade, Serbia

Abstract

Background/Aim. Mesenchymal stem cells (MSCs) have been shown to suppress immune and inflammatory reactions. However, it is not known whether MSCs from inflammatory tissues, such as periapical lesions (PLs) have similar effects. This question was addressed in this study in which the aim was to examine the capacity of PL-MSCs for modulating cytokine production by local immune cells. **Methods.** PL-MSCs were isolated from asymptomatic (as) and symptomatic (sy) PLs. Their phenotype was analyzed by flow cytometry by detecting MSC surface markers. Anti-inflammatory and immunomodulatory properties of PL-MSCs were examined by measuring cytokine production in direct co-culture experiments with mononuclear cells (MNCs) isolated from asPLs and syPLs, respectively. The levels of cytokines in supernatants were determined by specific ELISA kits. **Results.** Both PL-MSCs lines were characterized by typical MSC phenotype, with the predominance of CD29, CD44, CD90, CD105 and CD166. However, the lines, independently of their similar phenotype had the same modulatory effect on cytokine production, but the response of asPL-MNCs and syPL-MNCs was different, in spite of similar composition of these MNCs. Both MSC lines inhibited the production of inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor- (TNF-). However, IL-8 was only down-regulated in the co-culture of these MSC lines with syPL-MNCs. The PL-MSCs also modulated the production of immunoregulatory cytokines. Transforming growth factor- (TGF-) was up-regulated by both as- and syPL-MNCs but IL-10 was up-regulated only by asPL-MNCs. **Conclusion.** Our results showed that PL-MSCs contribute to the restriction of local inflammatory and immune responses, but this effect is probably less efficient during the exacerbation of PL inflammation.

Key words:
periapical diseases; stem cells; periapical abscess; inflammation; cytokines; phenotype; flow cytometry.

Apstrakt

Uvod/Cilj. Pokazano je da mezenhimske matične ćelije (engl. *mesenchymal stem cells* – MSC) suprimiraju imunske i inflamacijske reakcije. Medutim, nije poznato da li MSC iz inflamacijom zahvaćenih tkiva, kao što su periapeksne lezije (engl. *periapical lesions* – PLs) ispoljavaju slične efekte. Upravo je ovo pitanje razmatrano u našem radu čiji cilj je bio da se ispita sposobnost PL-MSC da moduliraju produkciju citokina od strane imunskih ćelija lokalno. **Metode.** MSC su izolovane iz asimptomatskih (engl. *asymptomatic* – as) i simptomatskih (engl. *symptomatic* – sy) PL zuba. Njihov fenotip je odreden metodom protočne citofluorimetrije na osnovu detekcije površinskih markera specifičnih za MSC. Antiinflamacijska i imunomodulacijska svojstva PL-MSC ispitivana su merenjem produkcije citokina u kokulturi sa mononuklearnim ćelijama (engl. *mononuclear cells* – MNCs) izolovanim iz asPLs i syPLs. Nivo produkovanih citokina u supernatantima odredivan je ELISA metodom. **Rezultati.** Obe PL-MSC linije karakterisao je fenotip tipičan za druge tipove MSC u kome je dominirala ekspresija CD29, CD44, CD90, CD105 i CD166 markera. Izolovane PL-MSC linije su, nezavisno od fenotipske sličnosti, ispoljile isti modulacijski efekat na produkciju citokina, ali je odgovor asPL-MNCs i syPL-MNCs bio različit, uprkos sličnom sastavu MNCs u oba tipa lezija. Oba tipa MSC li-

Correspondence to: Miodrag Čolić, University of Defence in Belgrade, Medical Faculty of the Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: mjcolic@eunet.rs

nija inhibirala su produkciju proinflamacijskih citokina kao što su interleukin 1 (IL-1) i faktor nekroze tumora (TNF-). Međutim, produkcija IL-8 bila je snižena jedino u kokulturi sa syPL-MNCs. PL-MSC linije su takođe modulirale produkciju imunoregulacijskih citokina. Produkcija transformišućeg faktora rasta (TGF-) bila je povećana u kokulturama sa oba tipa MNC, i asPL-MNCs i syPL-MNCs, a nivo IL-10 bio je povećan jedino u kokulturi sa asPL-MNCs. **Zaključak.** Naši rezultati pokazuju da PL-MSC doprinose smanjenju lokalne inflamacije i imunskog odgovora, ali je ovaj efekat verovatno manje efikasan u toku egzacerbacije inflamacije u PLs.

Ključne reči:
periapikalne bolesti; ćelije, matične; apsces, periapikalni; zapaljenje; citokini; fenotip; citometrija, protočna.

Introduction

Dental and periodontal tissues are a significant reservoir of mesenchymal stem cells (MSCs), which have been extensively investigated as a potent tool for the regenerative medicine [1, 2]. This hypothesis is based on relatively easy availability of these cells, their rapid propagation in culture, possibility to differentiate in different types of cells (osteoblasts, odontoblasts, chondroblasts, fibroblasts, adipocytes, neuronal cells) and other cells of mesenchymal origin [3, 4]. In addition, MSCs possess anti-inflammatory and immunosuppressive properties, making them usable for the treatment of chronic inflammatory and autoimmune diseases [5, 6].

Recently, MSCs have been isolated from inflamed dental and periodontal structures such as dental pulp [7], and periodontal ligament [8]. We have also established several MSCs lines from periodontal lesions and showed that these cells had many phenotypic similarities with MSCs from healthy tissue, such as dental pulp and dental follicle [9, 10]. However, it remained unclear whether periapical lesions (PL)-MSCs have similar functions as their counterpart from healthy periapical dental tissue, bearing in mind that PLs are chronic granulomatous pathoses [11]. It is well-known that PLs are triggered by bacterial infection from necrotic dental pulp and that these are characterized histologically by the presence of inflammatory infiltrate, mainly composed of mononuclear cells (MNCs) [12, 13]. In clinically asymptomatic (as) PLs, a balance between proinflammatory/osteodestructive and anti-inflammatory/osteoprotective processes is established [12–14]. The inflammatory processes are frequently exacerbated by the new wave of bacterial invasion, which is followed by pain, swelling and other clinical symptoms of infection. Histologically, such symptomatic (sy) PLs are characterized by the influx of neutrophil granulocytes within already established granulomatous tissue [12, 13, 15].

Based on our previous results, showing that dental MSCs suppress the immune response mediated by dendritic cells (DC), but that they may stimulate immune reactions under certain conditions [16], we wondered is there a difference between the capacity of PL-MSCs established from syPLs and asPLs, respectively, to influence the local production of cytokines. We hypothesized that different inflammatory cell substrate in syPLs and asPLs [15] is associated with different cytokine production. Therefore, to standardize the experimental conditions, we isolated MNCs from clinically different PLs and established an *in vitro* model by co-culturing these cells with asPL-MSCs or syPL-MSCs. We showed for the first time, that PL-MSCs, independently of its origin, are able to down-regulate the production of pro-inflammatory cytokines and up-regulated immunomodulatory cytokines by PL-MNCs. However, their immunomodulatory activity was lower during exacerbation of PL inflammation.

Methods

Donors and periapical tissues

A total of 10 PLs, 5 sy PLs and 5 as PLs, were collected from patients at the Department for Oral Surgery, Clinic for Stomatology, Military Medical Academy (MMA), Belgrade, during tooth extraction or apical surgery. Sy PLs and asPLs were classified according to the presence or absence of clinical symptoms [15]. The donors (range, aged 22–56 years) were without systemic diseases and had radiographic evidence of PLs. The donors have not been treated with antibiotics for 2 month before the surgery. No distinctions between samples were made regarding the etiology or the tooth type. One of specimens from asPLs and one from syPLs were used for the establishment of MSCs lines and the rest was used for preparation of inflammatory cells (ICs). The study was approved by the Ethical Committee of MMA, and informed consents were obtained. After collection, the tissue was placed into medium consisting of RPMI 1640 (Sigma, Munich, Germany) and antibiotics (gentamycine/penicillin/ streptomycin, 1% each) and transported to the laboratory.

Establishment of PL-MSC lines

PLs were excised by the curettage of firmly attached periodontal tissue from dental radices with a scalpel at the time of teeth extraction. Briefly, PLs tissues were digested in a Minimum Esential Medium (MEM, α-modification; Sigma, Munich, Germany) solution with a type I collagenase (1mg/mL; Sigma) and DNAase (25mg/mL; Sigma) for 1 h in 0.5% CO_2 atmosphere in an incubator with 5% CO_2. The cells were cultured in standard medium composed of α-MEM supplemented with 10% foetal calf serum (FCS; Sigma), 2-mercaptoetanol (2-ME) (Sigma), L-aspartat-2-phosphate (Sigma) and penicillin and streptomycin antibiotics 1% each (Galenika, Belgrade, Serbia). Passaging was performed by using equal volumes of 0.02% trypsin (Sigma) and 0.02% of Na-ethylenediaminetetraacetic acid (EDTA) dispersed in phosphate-buffered saline (PBS) solution. PL-MSCs used in the experiments were from the 4th passage.

Preparation of mononuclear cells

Inflammatory cells were isolated from asPLs and syPLs by a classic enzyme (collagenase/DNAase) digestion procedure, previously optimized at the Institute for Medical Research, MMA[17]. The total number of cells per lesion was in the range between 8.6×10^5–2.2×10^6. MNCs were prepared from PL-ICs by using LymphoPrep gradient (Nycomed, Oslo, Norway) and counted. Cytospins of both PL-ICs and PL-MNCs were stained with May-Grünwald Giemsa (MGG) and analysed by light microscopy. Identification of cell subsets on cytospins was made by clear morphological criteria. The term „other cells", was used for morphologically unidentified cells, including some blast-like cells and apoptotic cells. On each cytospin at least, a total of 500 cells were counted and particular cell subsets were determined. The results were presented as percentages of cell subsets relative to a total number of nucleated cells [17].

Phenotypic characterization of PL-MSC lines

For characterization of PL-MSC lines, the flow cytometry was used. The cells were stained with the following monoclonal antibodies (mAbs) conjugated with fluorescein isothiocyanate (FITC): anti-CD29, -CD105, -CD44, -CD19, -CD14, -CD45 (Immunotools, Friesoythe, Germany), -CD146, -CD46, -CD166, -CD90 (Serotec, Kidlington, UK). The indirect labelling was performed using anti-STRO-1 (Millipore/Chemicon) mAb, followed by secondary anti-mouse IgG1-FITC mAb (Serotec). During the staining procedure, the adequate isotype controls were set and the samples were acquired with a flow cytometer (Partec, CyFlow® Cube 6, Germany), as previously described [9].

Co-culture experiments

The effect of as- or syPL-MSCs on the production of cytokines by as- and syPL-MNCs were evaluated in the co-culture by using direct cell-to-cell contacts. PL-MNCs (1×10^5 cells/well) were cultivated with PL-MSCs (1×10^4/well) in triplicates, using 96-well plates for 48 h in the presence of phorbol myristate acetate (PMA; 20 ng/mL; Sigma) and Ca^{2+} ionophore (A23187, 1 μM; Sigma) during the last 8 h. Separate cultures of corresponding PL-MNCs and PL-MSCs, treated identically, were used as control. The levels of cytokines, produced in the PL-MSCs/PL-MNCs co-cultures, were compared with the sum of cytokines produced from separate PL-MNCs and PL-MSCs cultures. Each PL-MSC line was co-cultivated with four different as- or syPL-MNCs to evaluate cytokines production.

Cytokine detection

Concentrations of interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), IL-10 and transforming growth factor β1 (TGF-β1) referred as TGF-β, from the culture supernatants were determined using the commercial ELISA kits (R&D Systems), following instructions of the manufacturer. The standard curves were set up based on known concentrations of the cytokines.

Statistical analysis

Results are presented as mean ± SD. Mann-Whitney test was used to investigate the differences between the experimental and corresponding control samples. Values at $p < 0.05$ or less were considered to be statistically significant.

Results

Flow cytometric analysis of PL-MSC lines, established from asPLs and syPLs, showed very similar phenotypic properties (Table 1). Most cells expressed CD90, CD44, CD105, CD166 and CD29. A half of the cells expressed CD46 and CD146, whereas STRO-1 was detected on a subset of MSC (9.3% of asPL-MSC and 13.2% of syPL-MSC, respectively).

Table 1
Phenotypic characteristics of 2 mesenchymal stem cell (MSC) lines established from human periapical lesions (PL)

Markers	Expression (%)	
	asPL-MSC	syPL-MSC
CD90	99.8	97.4
CD44	99.4	95.9
CD29	98.8	97.4
CD166	93.6	94.2
CD105	98.3	96.1
CD46	57.0	50.3
CD146	50.4	42.4
STRO-1	9.3	13.2

PL-MSCs were prepared for the flow cytometry analysis, as described in the methods section. The labeled cells were gated according to the forward scatter/side scater parameters and the percentage of positive cells for the indicated markers were calculated based on the isotype control. Results are presented as % of positive cells. Standard deviation between duplicates was not higher than 2%.

as – asymptomatic; sy – symptomatic.

To study how these MSC lines modulate local cytokine production we established an *in vitro* model by co-cultivating PL-MSC lines and MNCs prepared from either as- or syPL-ICs. As presented in Table 2, syPLs differed from asPLs by higher proportion of granulocytes and lower percentages of lymphocytes. After removal of granulocytes by density gradient, no significant differences in the cellular composition of MNCs between asPLs and syPLs were observed (Table 3). The MNC subsets composed predominantly of lymphocytes, followed by almost equal proportion of plasma cells and macrophages (MØ), whereas the percentage of DC was the lowest.

The analysis of cytokines from the co-cultures showed that all examined cytokines were produced by PL-MNCs, but very little or none by MSC lines. MSC lines, independently of their origin, exerted the same modulatory effect on cytokine production by PL-MNCs. However, the response of asPL-MNCs and syPL-MNCs was different.

Table 2
Cellular composition of a total of inflammatory cells (ICs) isolated from human periapical lesions (PL)

Cell subsets	asPL-ICs (%)	syPL-ICs (%)
GR	14.2 ± 5.3	47.6 ± 8.7***
LY	46.5 ± 10.8	22.5 ± 6.9**
PC	19.3 ± 8.1	10.4 ± 3.2
MØ	15.9 ± 3.4	11.3 ± 3.1
DC	2.6 ± 1.3	2.2 ± 0.6
Other cells	3.1 ± 3.1	6.0 ± 3.3

Inflammatory cells were isolated from asymptomatic and symptomatic perapical lesions (both n = 4), as described in the section metods. Cytospins were stained with May-Grünwald Giemsa and analysed by light microscopy. The percentages of cell subsets were determined based on morphological criteria after calculation of 500 cells on each cytospin. Values are given as mean ± standard deviation (SD) (n = 4). ** $p < 0.01$, *** $p < 0.001$ compared to corresponding controls. GR – granulocytes; LY – lymphocytes; PC – plasma cells; MØ – macrophages; DC – dendritic cells; as – asymptomatic; sy – symptomatic.

Table 3
Cellular composition of mononuclear cells (MNSc) isolated from human periapical lesions (PL)

Cell subsets	asPL-MNCs (%)	syPL-MNCs (%)
GR	3.1 ± 1.3	1.7 ± 1.2
LY	43.3 ± 8.1	48.8 ± 9.3
PC	21.4 ± 9.1	21.2 ± 6.7
MØ	23.8 ± 3.9	21.2 ± 3.5
DC	4.5 ± 0.8	3.5 ± 1.6
Other cells	4.0 ± 2.7	3.7 ± 2.7

PL-MNCs were prepared from inflammatory cells isolated from asymptomatic and symptomatic periapical lesions (both n = 4), as described in the section metods. Cytospins were stained with May-Grünwald Giemsa and analysed by light microscopy. The percentages of cell subsets were determined based on morphological criteria after calculation of 500 cells on each cytospin. Values are given as mean ± standard deviation (SD) (n = 4). No statistical significant differences in any cell subset were observed among the groups. GR – granulocytes; LY – lymphocytes; PC – plasma cells; MØ – macrophages; DC – dendritic cells; as – asymptomatic; sy – symptomatic.

Namely, both MSC lines inhibited the production of proinflammatory cytokines (IL-1β and TNF-α) by as- and syPL-MNCs (Figures 1 and 2). In contrast, IL-8 was down-regulated only in the co-culture of PL-MSC lines with syPL-MNCs (Figure 3).

The PL-MSC lines were up-regulated the production of immunoregulatory cytokines by asPL-MNCs (IL-10 and TGF-β) and syPL-MNCs (TGF-β) (Figures 4 and 5). However, the levels of IL-10 in the co-cultures between PL-MSC lines and syPL-MNCs were not significantly modulated.

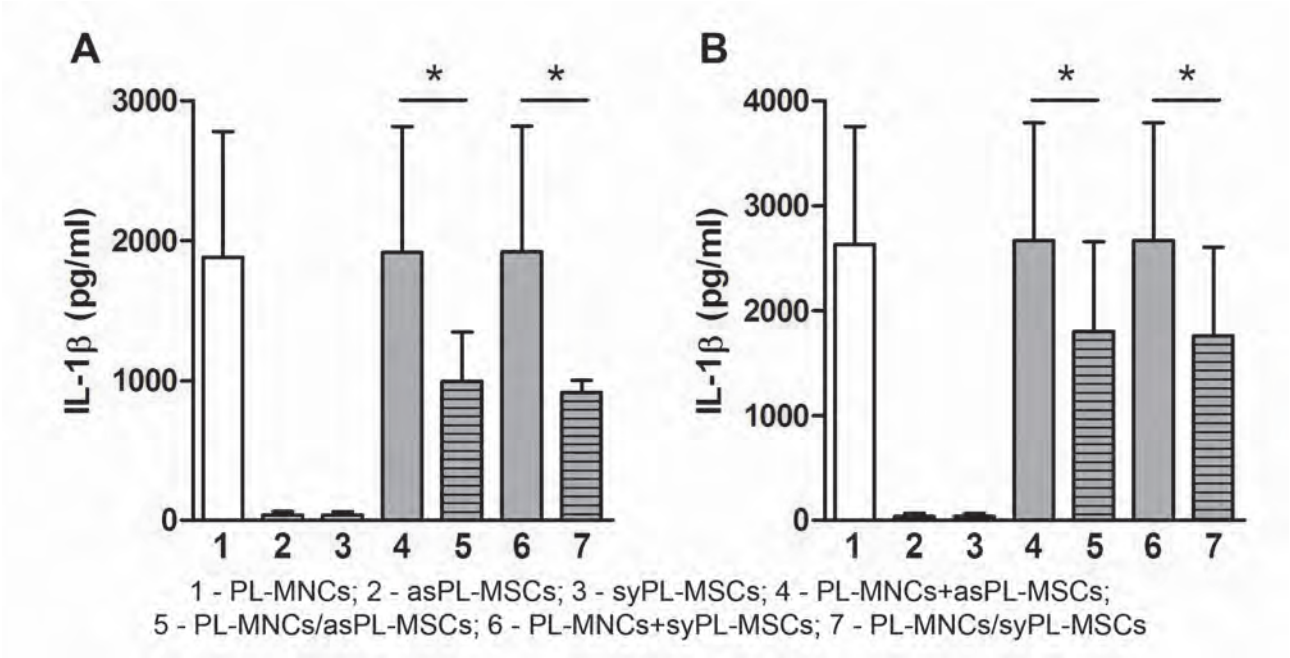

Fig. 1 – The effect of PL-MSCs on the production of IL-1β by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs. B) Co-culture PL-MSCs with syPL-MNCs. Values are given as mean ± SD (n = 4).*$p < 0.05$ compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).
PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

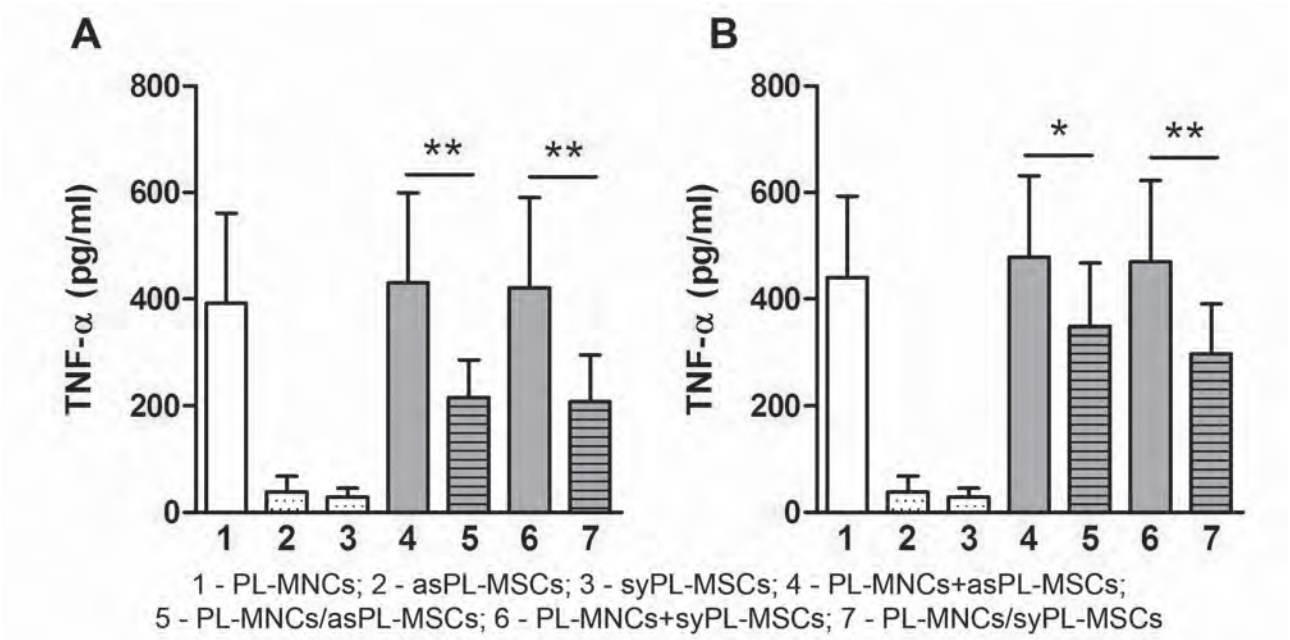

Fig. 2 – The effect of PL-MSCs on the production of TNF-α by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs. B) Co-culture PL-MSCs with syPL-MNCs.
Values are given as mean ± SD (n = 4).*$p < 0.05$, **$p < 0.01$ compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).
PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

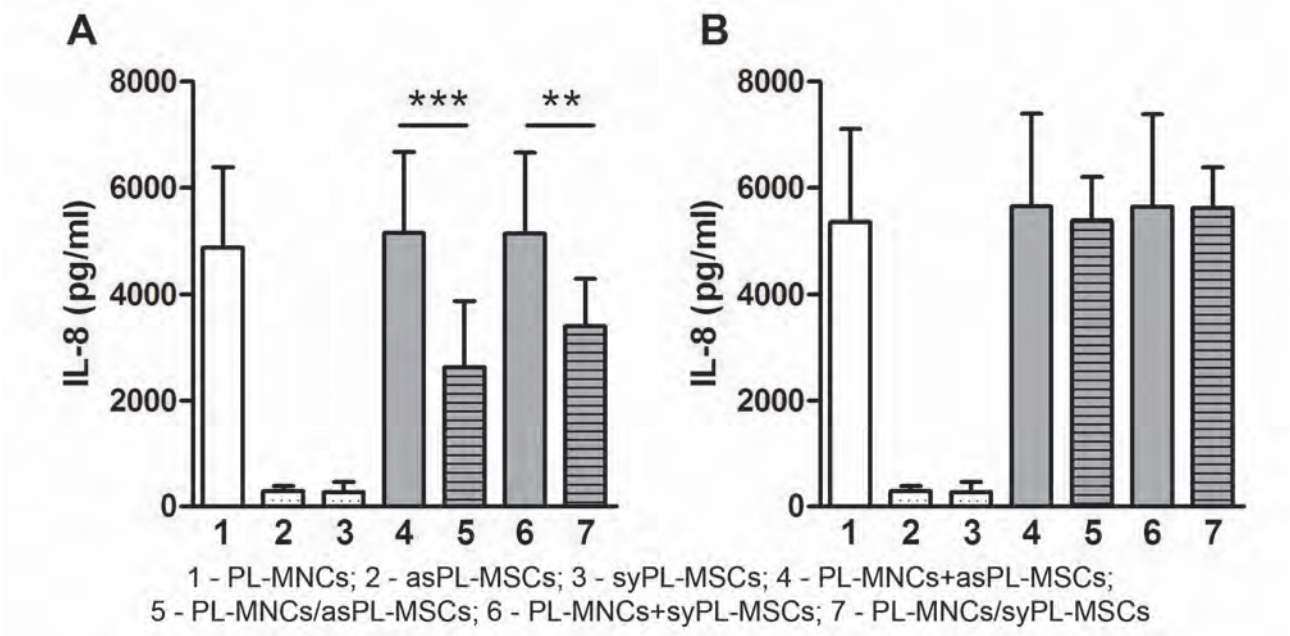

Fig. 3 – The effect of PL-MSCs on the production of IL-8 by PL-MNCs in co-culture. PL-PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs. B) Co-culture PL-MSCs with syPL-MNCs.
Values are given as mean ± SD (n = 4).$p < 0.01$, ***$p < 0.001$ compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).**
sy – symptomatic; as – asymptomatic.

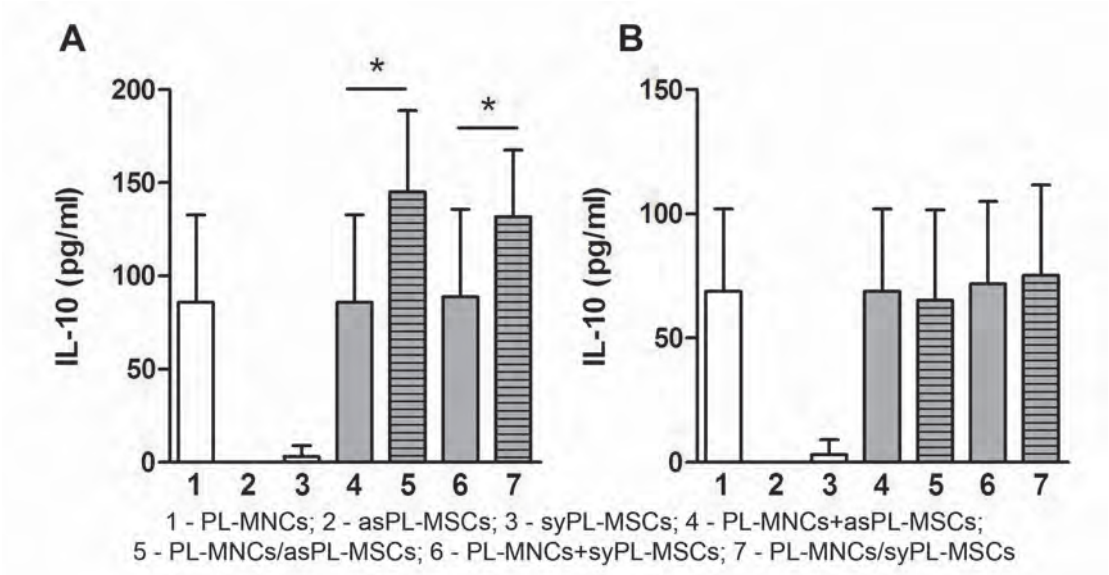

Fig. 4 – The effect of PL-MSCs on the production of IL-10 by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs. B) Co-culture PL-MSCs with syPL-MNCs.
Values are given as mean ± SD (n = 4).*$p < 0.05$ compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).
PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

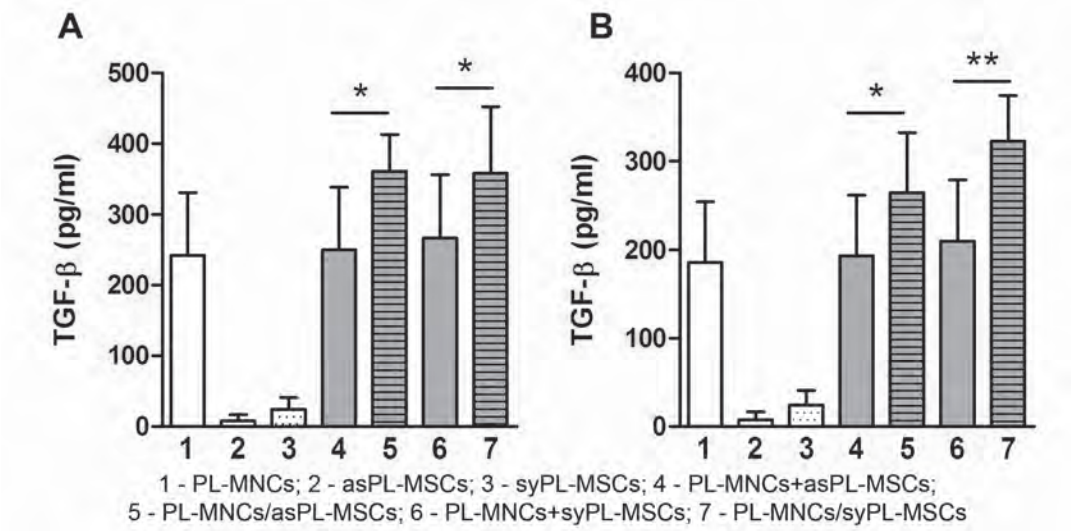

Fig. 5 – The effect of PL-MSCs on the production of TGF-β by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). Separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs. B) Co-culture PL-MSCs with syPL-MNCs.
Values are given as mean ± SD (n = 4).*$p < 0.05$, **$p < 0.01$ compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).
PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

Discussion

This study, together with our previous results [9, 16, 18], presents the rare publication dealing with characterization of MSCs from inflamed dental/periodontal tissues [7, 8]. We showed that PL-MSC lines possess all properties similar to other MSCs, including typical phenotype [19], capability to proliferate and form colonies *in vitro* as well as to differentiate into osteoblasts, chondrocytes and adipocytes [7, 9, 20]. The phenotypic analysis of new established PL-MSC lines demonstrated the expression of high levels of CD29, CD44, CD90, CD105 and CD166, moderate expression of CD46 and CD146 and low expression of STRO-1, regardless weather PLs were asymptomatic or symptomatic. This phenotype is similar to our previous finding with PL-MSCs, generated from several asPLs [9].

It is known that PLs are heterogenous in terms of their cellular composition [15], because PL development and persistence are a dynamic process in which many host and microbial factors play a role [12]. We confirmed here that asPLs differ from syPLs by higher proportion of lymphocytes and plasma cells and lower percentages of granulocytes [15]. Such cellular composition is associated with the established chronicity, where anti-inflammatory mediators predominate (asPLs), or with the exacerbation of inflammation, followed by up-regulation of proinflammatory and osteo destructive mechanisms (syPLs). Due to these differences, we postulated that PL-MSCs from clinically different PLs may have different influence on PL-MNCs and that, due to the same reasons, MNCs from such PLs respond differently to the action of the PL-MSCs. Such hypothesis has not been tested so far. To prove this, we established a co-culture assay by incubating PL-MSC lines from different sources with as- and syPL-MNCs and measured cytokine production. The cultures were additionally treated with PMA/Ca^{2+} ionophore, as a recommended approach to enhance the cytokine production *in vitro*[21]. These MNCs had a very similar composition and this was the reason why they were used instead of total, very heterogenous, PL-ICs.

Some of examined cytokines were produced also by PL-MSCs, themselves, but their levels were significantly lower than in cultures with PL-MNCs due to much lower number of MSCs. Therefore, to avoid any mistake in conclusion, we compared the levels of cytokines in PL-MSCs/PL-MNCs co-culture with the sum of cytokines produced in separate cultures.

Generally, our results showed that both PL-MSC lines had similar modulatory activity on cytokine production, but the response of MNCs was different. Suppression of TNF-α and IL-1β production was an expected phenomenon, shown already for other MSCs and it is in accordance with well-proven anti-inflammatory properties of MSCs [22, 23]. This finding also suggests that local MSCs in PLs could control an excessive inflammation and, in this context, their clinical use may be beneficial for the restriction of inflammation-induced osteo destructive processes and the reparation of local tissues. It is known that both TNF-α and IL-1β, through their action via specific receptors on infiltrating and stromal cells in PLs, stimulate inflammation in order to resolve infection. Additionally, the cytokines, indirectly by acting on the receptor activator of nuclear factor kappa-B (RANK)/RANK-ligand (L) signalling pathway, promote osteolytic processes, a hallmark of periodontal diseases, including apical periodontitis [24].

It seems that the regulation of IL-8 by PL-MNCs was different. Namely, although this chemokine was down-regulated by asPL-MNCs, similarly as we have already shown by total PL-ICs [9], the production of IL-8 was not significantly changed by syPL-MNCs. It is known that IL-8 is a neutrophil recruiting chemokine [25] and its secretion is enhanced by IL-17 [26]. Our previous results [15, 26] demonstrated that IL-17 is one of the key cytokines responsible for the exacerbation of inflammation in PLs. Furthermore, we showed that PL-MSCs were not able to down-modulate significantly the production of IL-17 by both periapical blood MNCs and PL-ICs [9], which could explain the absence of IL-8 down-regulation by syPL-MNCs observed in this study.

It is obvious that, although the composition of MNCs from syPLs and asPLs are similar, their functional state and subset content, especially within T cells [15], is different. Such observations are also supported by the modulatory effect of PL-MSCs on the IL-10 production in the co-cultures. The production of this cytokine was augmented by asPL-MNCs but this was not the case with syPL-MNCs. It is well-known that IL-10, as a key immunoregulatory cytokine, suppresses the production of cytokines by T helper (Th)1, Th2 and Th17 cells as well as IL-6, IL-1β, IL-8, IL-12 and many others [27]. T regulatory cells (Tregs) are the main source of IL-10 [27] and this finding was also confirmed for PLs in our previous papers [28]. Since the counterbalance between Tregs and IL-17 exists [28, 29], it can be postulated that these interactions are less pronounced in syPLs. This is another fact supporting the hypothesis that the suppression of inflammation/immune response by PL-MSCs during the exacerbation of inflammation is not a desirable mechanism. Therefore, it will be of interest to know which factors control the down-modulatory functions of PL-MSCs under such conditions.

However, PL-MSCs were able to up-regulate another immunoregulatory cytokine, TGF-β, by both asPL-MNCs and syPL-MNCs. This cytokine is produced by leukocytes, macrophages, fibroblasts, osteoblasts and, most importantly Tregs [30]. TGF-β stimulates migration of lymphocytes and monocytes to the inflammatory site, but inhibits their proliferation subsequently[31]. In addition, together with IL-10, it suppresses the production of different pro-inflammatory cytokines [30, 31]. TGF-β is also expressed in PLs [15, 32], irrespective of clinical presentation of PLs, and represents a key down-modulator of inflammation and osteodestructive processes. In this context, it is not only that PL-MSCs produce TGF-β, but also stimulate its production by other cells in PLs.

Conclusion

Cumulatively, our results showed that PL-MSCs could contribute substantially to the restriction of local inflammation and immune response during chronic, asymptomatic course of PLs development by down-regulating the expression of pro-inflammatory cytokines and up-regulating immunoregulatory cytokines. It can be postulated that during exacerbation of inflammation within PLs, PL-MSCs allow the recruitment of granulocytes and maybe other cells, through the balance between IL-17/IL-10 axis and IL-8. However, the cells are still potent to control excessive inflammation through down-regulation of IL-1β and TNF-α and up-regulation of TGF-β. Further studies are needed to understand how these processes are controlled.

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Changes in maximal oxygen uptake during growth and development in girls who actively participate in basketball and non-athletes girls: a longitudinal study

Promene u maksimalnoj potrošnji kiseonika tokom rasta i razvoja devojčica koje igraju košarku i devojčica koje se ne bave sportom: longitudinalna studija

Tamara Stojmenović*, Djordje Ćurčić*, Milica Vukašinović-Vesić*, Marija Andjelković*, Nenad Dikić*, Marija Kostić-Vučićević*, Ivana Baralić*, Vladimir Jakovljević†, Vladimir Živković†

***Sports Medicine Association of Serbia, Belgrade, Serbia; University of Kragujevac, Faculty of Medical Sciences, †Department of Physiology, Kragujevac, Serbia**

Abstract

Background/Aim. It is well known that continuous engagement in physical activity is important for normal growth and development of children. Maximal oxygen uptake (VO_{2max}), as a measure of functional state of the organism, is largely affected by level of physical activity, but it remains unclear to what extent it can be improved during childhood. The aim of the study was to evaluate dynamics of changes in aerobic capacity, anthropometric and body composition characteristics in active and non-active girls over a period of 3 years. **Methods.** A total of 48 young girls were included in the study. Girls were divided into 2 groups: training group consisted of 25 girls who played basketball (age 13.84 ± 0.94) and non-training group of 23 girls who were not involved in any organized sports (age 13.83 ± 0.98). Anthropometric and body composition characteristics were measured in order to monitor somatic growth during the study. VO_{2max}values were obtained by performing cardiopulmonary exercise testing on a treadmill. All parameters were measured every 6 months during 3-years period. **Results.** ANOVA analysis showed a significant time and group interaction effect on VO_{2max} ($p < 0.001$), body mass index (BMI) ($p < 0.001$) and fat percentage (FAT%) ($p < 0.01$). Also, there was an obvious increase in VO_{2max} within both groups due to growth and development itself ($p < 0.001$). **Conclusion.** The main finding of the study was an increase in VO_{2max} due to growth and development. The girls who actively participated in basketball had higher level of aerobic capacity compared to non-active girls. Furthermore, continuous basketball training led to maintaining normal body composition in terms of FAT% and BMI, which altogether may imply that organized physical activity has a positive influence on evaluated characteristics.

Key words:
exercise; oxygen consumption; growth and development; basketball; adolescent; women.

Apstrakt

Uvod/cilj. Poznato je da je redovna fizička aktivnost važna za normalan rast i razvoj dece. Maksimalna potrošnja kiseonika (VO_{2max}), kao mera funkcionalnog stanja organizma, u velikoj meri zavisi od nivoa fizičke aktivnosti, ali nije jasno u kojoj meri ova vrednost može biti unapređena tokom detinjstva. Cilj rada bilo je praćenje dinamike promene aerobne sposobnosti i antropometrijskih karakteristika devojčica koje treniraju košarku i onih koje se ne bave sportom u periodu od tri godine. **Metode.** U studiji je učestvovalo 48 devojčica podeljenih u dve grupe: grupa od 25 devojčica (uzrasta 13,84 ± 0,94) koje su trenirale košarku i grupa od 23 devojčice (uzrast 13,83 ± 0,98) koje se nisu bavile sportom. U cilju praćenja somatskog rasta tokom studije vršena su antropometrijska merenja i procena telesne kompozicije. Vrednosti VO_{2max} dobijene su izvođenjem maksimalnog ergospirometrijskog testa opterećenja pokretnoj traci. Svi parametri mereni su na svakih 6 meseci tokom perioda od 3 godine. **Rezultati.** ANOVA analiza pokazala je značajan efekat interakcije između vremena studije i grupa u pogledu VO_{2max} ($p < 0.001$), indeksa telesne mase (BMI) ($p < 0.001$) i procenta telesne masti (FAT%) ($p < 0.01$). Takođe, uočen je i očigledan porast VO_{2max} u obe grupe tokom studije kao rezultat rasta i razvoja ($p < 0.001$). **Zaključak.** Glavni zaključak studije je porast u VO_{2max} kao posledica rasta i razvoja. Takođe, redovna fizička aktivnost omogućila je održavanje normalne telesne kompozicije u pogledu BMI i FAT%, što sve zajedno može da implicira da organizovana fizička aktivnost ima pozitivan uticaj na praćene parametre.

Ključne reči:
vežbanje; kiseonik, potrošnja; rast i razvoj; košarka; adolescenti; žene.

Correspondence to: Tamara Stojmenović, Sports Medicine Association of Serbia, Maršala Tolbuhina 8, 11 000 Belgrade, Serbia.
E-mail: antictamara@hotmail.com

Introduction

Physical activity is an important factor for normal growth and development of children since continuous exercise enhances the functional state of entire organism and has beneficial effects on body composition and basic motor skills [1]. It is essential to adopt proper habits in terms of physical activity during childhood in order to prevent sedentary lifestyle and, consequently, all the concomitant diseases (cardiovascular diseases, type 2 diabetes, obesity, etc.) [1–4].

Aerobic capacity represents the functional state of the entire organism, but largely depends on the ability of the heart and lung to deliver an adequate amount of oxygen to our muscles in order to produce energy for work. Maximal oxygen uptake (VO_{2max}) is the measure of aerobic capacity and cardiorespiratory fitness per se. It is significantly affected by genetic factors, gender, age, and level of physical activity. Although it is predominantly genetically determined, active participation in sports can lead to full expression of genetic potential and subsequently to an increase in aerobic capacity, especially in period between age 18 and 25 [5]. On the other hand, VO_{2max} values are increasing during growth and development of children due to increase in body size, but it remains unclear to what extent aerobic capacity can be improved by engaging in continuous physical activity in such early and sensitive period of life [2, 3, 6, 7]. There are limited data available on changes in VO_{2max} during growth and development of both active and inactive children, especially longitudinal data are lacking for young girls.

Therefore, the study was designed to evaluate dynamics of changes in aerobic capacity, anthropometric and body composition characteristics in 2 groups of girls over a period of 3 years. The obtained results should provide the basis for a discussion on the individual influence of growth and development as well as basketball training on the evaluated parameters.

Methods

Subjects

A total of 48 young girls were included in the study. Girls were divided into 2 groups: the training group consisted of 25 girls who played basketball (age 13.84 ± 0.94 at the beginning of the study; age 16.32 ± 1.21 at the end of the study) and the non-training group of 23 girls who were not involved in any organized sports (age 13.83 ± 0.98 at the beginning of the study; age 16.65 ± 0.88 at the end of the study). The girls from the training group were recruited from 3 local basketball teams in Belgrade, Serbia. At the baseline of the study they have already played basketball 3.65 ± 1.67 years on average and had 5.7 ± 2.13 h of training per week. The non-training group consisted of girls who attended the same elementary schools as their peers in the training group. Both groups were homogeneous in terms of age, gender and socioeconomic status.Written informed consent was obtained through a letter given to parents explaining study goals, procedures, and methods. The protocol was in accordance with the Declaration of Helsinki for research on human subjects and was approved by the Ethical Committee of Sports Medicine Association of Serbia.

Measurements

Participants were evaluated at outpatient sports medicine clinic, under the auspice of the Sports Medicine Association of Serbia, every 6 months (April/October) during a period of 3 years (total of 6 tests were carried out).

Physical/somatic growth of the young girls was estimated by obtaining anthropometric and body composition characteristics. Height was measured by Seca stadiometer to the nearest 0.1 cm. Arm span was obtained by using tape measure technique. Tanita Body Composition Analyzer BC-418MA was used to measure body weight, body mass index (BMI), body fat percentage (FAT%), and free fat mass (FFM).

VO_{2max} values were obtained by performing cardiopulmonary exercise testing (CPET) on a treadmill (H-P-COSMOS). Before the CPET, participants passed sports medical examination and were eligible for carrying out maximal progressive test. The subjects were equipped with a face mask, heart rate monitor (COSMED Wireless HR Monitor) and ECG device (Quarck T 12x, Wireless 12-lead ECG) in order to perform the test. The initial speed and inclination were set at 2.5 km/h and 3°, respectively. Every 30 seconds treadmill speed was increased by 0.5 km/h, while the inclination remained constant throughout the test. The protocol was created, as described above, taking into account the age of participants and the fact that most of them did not use the treadmill before. A very low speed at the beginning of the test, with only 0.5 km/h of increase in 30 sec, has provided enough time to get familiar with the treadmill and walking and running technique. The treadmill inclination was set in order to amortize steps on a treadmill and avoid local muscle fatigue and aches. Oxygen consumption kinetics was measured continuously by using breath-by-breath analysis technique (Quark CPET system manufactured by Cosmed). Heart rate was monitored by both COSMED Wireless HR Monitor and ECG device. A test was considered maximal if participants achieved 90% or more of predicted maximal heart rate for age and gender. The 220 – age equation was used in order to calculate maximal heart rate predicted values (Tanaka, 2001). Furthermore, a plateau in oxygen consumption despite increased workload, a respiratory exchange ratio grater than 1.00 and reached volitional exhaustion were also criteria for the end of the test. All the tests were performed by trained personnel and the test equipment were routinely calibrated with both volume and gas calibration every fifth test.

Statistical analysis

Statistical analysis was performed with the software IBM SPSS Statistic version 20.0. All data were assessed for normality (one-sample Kolmogorov-Smirnov test). Paired samples *t*-test was used to compare measured characteristics within the groups at the beginning and the end of the study. The subject's baseline and final characteristics between the groups were compared using independent-sample *t*-test. A

comparison of continuous variables was performed by general linear model 2-way analysis of variance (ANOVA) with repeated measures, with group (research group vs. control group) as the between-subjects effects, and time (baseline vs. end of the observational period) as within-subjects effects. Descriptive statistics of data for both groups were given as r ± SD. A p-value ≤ 0.05 was considered statistically significant.

Results

The anthropometric and body composition characteristics for both groups are presented in Tables 1 and 2 respectively.

Within the group changes

Main effects of time on anthropometric and body composition parameters are shown in Table 2. A significant increase in FAT% was noticed in the non-training group after 3-years follow-up in comparison to baseline values (paired samples t-test, $p < 0.01$), while there were no significant changes in the training group in terms of this parameter (paired samples t-test, $p > 0.05$). Furthermore, Figure 1 shows an obvious increase in VO_{2max} values within the both groups throughout the study (ANOVA repeated measures; main effect of time, $p < 0.001$). In addition, Figure 2 and 3, respectively, present trend changes in BMI, FAT% and VO_{2max} values within the training and non-training group over time.

Between the group changes and time and group interaction effects on measured anthropometric and body composition parameters are shown in Table 2. At the baseline of the study, there was no differences between the active and inactive girls regarding FAT% ($p > 0.05$), but final measurements revealed significant difference in terms of this characteristic (independent sample t-test, $p < 0.01$). Furthermore, ANOVA analysis also showed a significant time and group interaction effect on VO_{2max} ($p < 0.001$).

Table 1

Anthropometric and body composition characteristics of the training group / non-training group during the study period

Parameters	Test I	Test II	Test III	Test IV	Test V	Test VI
	r ± SD	r ± SD	r ± SD	r ± SD	r ± SD	r ± SD
Height (cm)	169.3 ± 6.48/ 165.0 ± 7.39	170.1 ± 6.26/ 166.0 ± 6.89	170.6 ± 6.04/ 166.8 ± 6.93	171.2 ± 5.94/ 167.2 ± 6.71	171.6 ± 5.92/ 167.2 ± 6.72	172.0 ± 5.88/ 168.2 ± 6.30
Arm span (cm)	167.5 ± 8.20/ 163.6 ± 8.88	168.8 ± 7.96/ 164.0 ± 8.82	169.4 ± 7.62/ 165.4 ± 8.23	169.9 ± 7.97/ 166.0 ± 7.92	170.1 ± 7.97/ 166.0 ± 7.94	170.4 ± 7.84/ 166.6 ± 7.84
Weight (kg)	59.76 ± 7.67/ 56.52 ± 9.22	61.7 ± 7.63/ 58.19 ± 9.80	62.03 ± 7.30/ 59.25 ± 9.67	62.23 ± 6.60/ 60.27 ± 10.60	62.88 ± 6.51/ 60.32 ± 10.61	63.24 ± 6.58/ 61.74 ± 10.05
Body mass index (kg/m^2)	20.95 ± 2.50/ 20.59 ± 2.74	21.31 ± 2.67/ 21.13 ± 2.96	21.44 ± 2.55/ 21.17 ± 2.49	21.34 ± 2.35/ 21.98 ± 2.59	21.62 ± 2.35/ 22.07 ± 3.19	21.74 ± 1.98/ 23.05 ± 2.95
Body fat percentage (%)	25.38 ± 3.37/ 26.57 ± 4.35	25.53 ± 4.01/ 26.18 ± 4.63	25.81 ± 3.84/ 26.89 ± 4.47	25.0 ± 3.63/ 27.34 ± 4.97	25.64 ± 4.01/ 27.42 ± 4.98	25.28 ± 4.01/ 28.59 ± 4.21
Free fat mass (kg)	44.44 ± 4.82/ 41.32 ± 5.25	45.23 ± 5.18/ 42.53 ± 5.56	45.58 ± 4.48/ 43.02 ± 4.93	46.40 ± 4.22/ 43.78 ± 4.90	46.75 ± 4.52/ 43.84 ± 4.90	47.42 ± 4.15/ 44.69 ± 4.75

Test I – Test VI: performed measurements during period of 3 years on every 6 months (April/October).

Table 2

Within and between the group changes in terms of anthropometric and body composition characteristics and VO_2max values during the study

Parameters	Training group		Non-training group		ANOVA p values		
	baseline	final	baseline	final	time[a]	group[b]	time *group[c]
Height (cm)	169.3 ± 6.48	172.0 ± 5.88	165.0 ± 7.39	168.2 ± 6.30	< 0.001	< 0.05	ns
Arm span (cm)	167.5 ± 8.20	170.4 ± 7.84	163.6 ± 8.08	166.6 ± 7.84	< 0.001	ns	ns
Weight (kg)	59.76 ± 7.67	63.24 ± 6.58	56.52 ± 9.22	61.74 ± 10.05	< 0.001	ns	ns
Body mass index (kg/m^2)	20.95 ± 2.50	21.74 ± 1.98	20.59 ± 2.74	23.05 ± 2.95	< 0.001	ns	< 0.001
Body fat percentage (%)	25.38 ± 3.37	25.28 ± 4.01	26.57 ± 4.35	28.59 ± 4.21	< 0.05	ns	< 0.01
Free fat mass (kg)	44.44 ± 4.82	47.42 ± 4.15	41.32 ± 5.25	44.69 ± 4.75	< 0.001	< 0.05	ns
VO_2max (mL/kg/min)	41.62 ± 4.57	49.85 ± 4.20	35.13 ± 2.59	39.03 ± 2.66	< 0.001	< 0.001	< 0.001

[a]Time (baseline vs. end of the observational period) refers to within-subjects effects.
[b]Group (training vs. non-training group) refers to between-subjects effects.
[c]Time*group refers to time and group interaction effects.
VO_2max – maximal oxygen uptake.

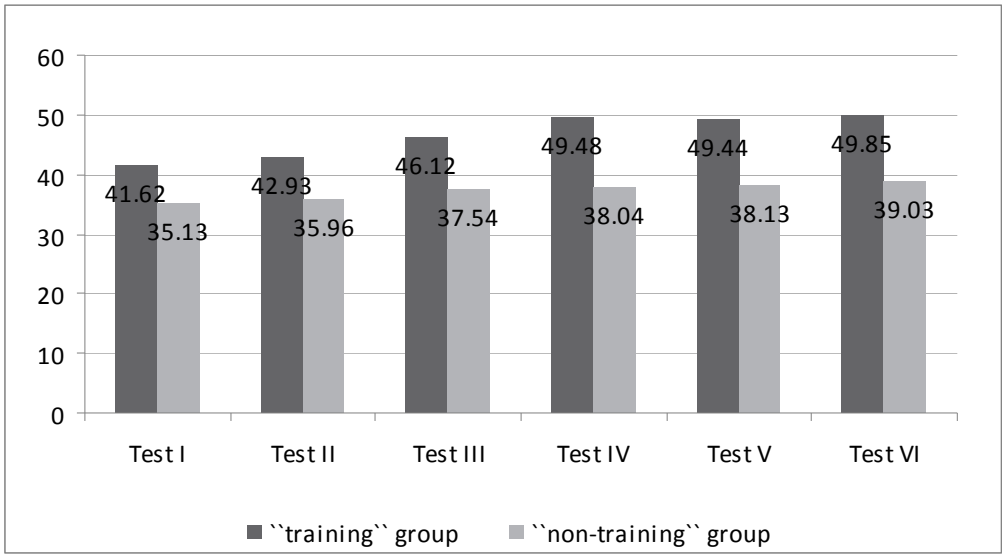
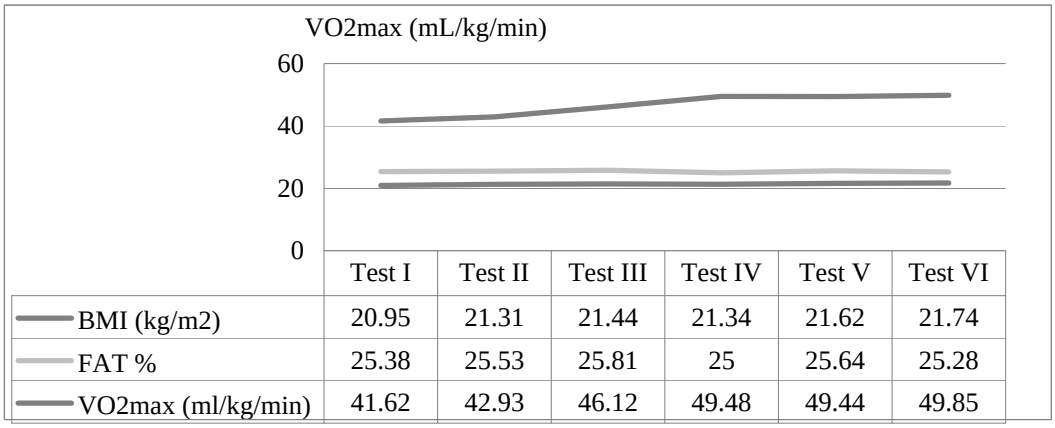
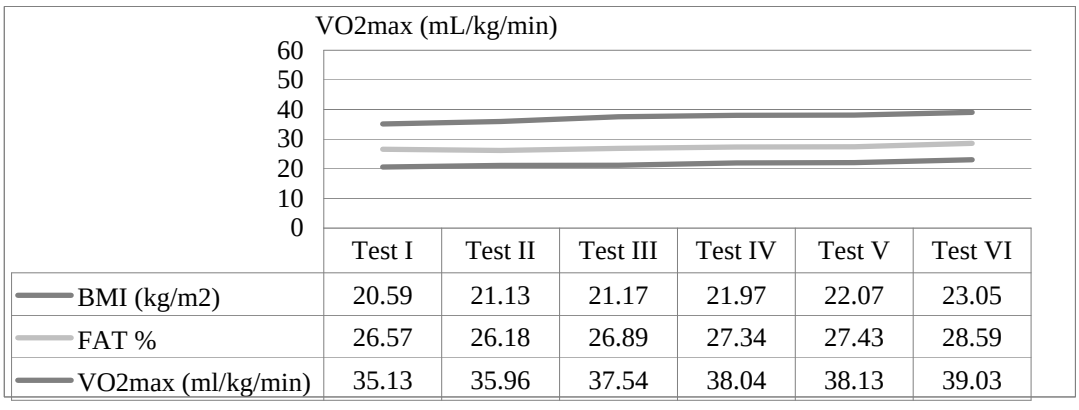


Fig. 1 – Changes in VO_{2max} values (mL/kg/min) in the training and non-training group measured on every 6 months over a period of 3 years.
VO_2max – maximal oxygen uptake.



	Test I	Test II	Test III	Test IV	Test V	Test VI
BMI (kg/m2)	20.95	21.31	21.44	21.34	21.62	21.74
FAT %	25.38	25.53	25.81	25	25.64	25.28
VO2max (ml/kg/min)	41.62	42.93	46.12	49.48	49.44	49.85

Fig. 2 – Trend changes in BMI, FAT% and VO_{2max} values within the training group over time.
BMI – body mass index; FAT – body fat percentage; VO2max – maximal oxygen uptake.



	Test I	Test II	Test III	Test IV	Test V	Test VI
BMI (kg/m2)	20.59	21.13	21.17	21.97	22.07	23.05
FAT %	26.57	26.18	26.89	27.34	27.43	28.59
VO2max (ml/kg/min)	35.13	35.96	37.54	38.04	38.13	39.03

Fig. 3 – Trend changes in BMI, FAT% and VO_{2max} values within the non-training group over time.
BMI – body mass index; FAT – body fat percentage; VO2max – maximal oxygen uptake.

Discussion

The main finding of the present study was an increase in VO_{2max} in both study groups over time. Furthermore, the participants from the training group achieved higher VO_{2max} values compared to their non-active peers.

Representative data of VO_{2max} values in the young girls were scarce, presented within a wide range of age (i.e. 13–19), and vary among studies depending on sample size and measurement methods [3, 8]. Our data are highly comparable with those reported in a study including 92 active and 75 non-active Spanish girls (41.85 mL/kg/min and 39.03 mL/kg/min in 12 to 18-years-old active and non-active girls, respectively) [4]. On the other hand, higher VO_{2max} values were obtained among Indian girls (54.4 ± 5.00 and 48.70 ± 3.21 ml/kg/min in 12 to 17-years-old active and non-active girls, respectively) [9] and yet, lower in 6 to14-years-old Serbian girls (30.4 ± 9.6 mL/kg/min) probably due to younger age of evaluated participants [10]. Compared for normative data for VO_{2max}, the training group girls were graded as excellent (39.0–41.9 mL/kg/min) at the beginning of the study and as superior (> 41.9 mL/kg/min) by the end of the observational period. Aerobic capacity of the non-training group was graded as good (35.0–38.9 mL/kg/min) throughout the entire study.

Earlier studies reported a gradual increase in VO_{2max} through childhood and adolescence due to growth, development, and a subsequent increase in body size and muscle mass, which enhances oxygen utilization. Accordingly, maturation, age, and body composition may account for most of differences in VO_{2max} values in pre-pubertal and pubertal children [3, 11], with only weak to moderate training-induced increase inVO_{2max} during this sensitive period of life [3, 7, 8, 11]. In line with our findings, a gradual increase in aerobic capacity goes in favor to growth and development. The continuous increase in body height, arm span, body weight, BMI and FFM over time clearly contributed to better oxygen utilization and an increase in VO_{2max} values. On the other hand, the recent research on this matter [7, 9, 12, 13] showed a strong association between regular engagement in physical activity and aerobic capacity in children, which might explain higher VO_{2max} values in training group compared to non-training one. The higher level of aerobic capacity in the trained group of girls could be associated with an increased respiratory volumes, stroke volume and cardiac output, which are in favor of both morphological and functional adaptations of heart and lungs to organized and continuous physical activity [7].

Furthermore, being involved in regular basketball training led to maintaining normal body composition in terms of FAT% and BMI during growth and development [10]. It is well known that lower FAT%, with normal BMI and greater FFM are also correlated with higher cardiorespiratory fitness [14]. In addition, athletic training at a non-elite level did not affect growth in somatic sense [15]. Growth spurt and changes in weight were the same for both groups, since there was no significant time and group interaction effect on these parameters by the end of the observational period.

In addition, according to Robbins et al. [15], by the age of 13, only about 22% of girls meet World Health Organization recommendations of at least 60 min of physical activity every day [1, 16, 17] and almost 28% are already overweight or obese. These findings indicate that young girls would benefit from continuous involvement in exercise programs, such as those designed to increase physical activity [15, 18, 19]. The obtained results could be in a favor of positive effects of physical activity on aerobic capacity, body composition and consequently cardiorespiratory system of young girls itself.

Limitations

Although the present study has certain advantages such as direct measurement of VO_{2max} and evaluation of a large number of parameters over a period of 3 years, it also has some limitations. At the beginning of the study there was already statistically significant difference in terms of $VO_{2max,}$ body weight and FFM between the two evaluated groups. The complex interaction between basketball training and growth and development could be properly evaluated only if there were no significant differences between the groups in terms of baseline measurements. The results obtained by this comparative longitudinal study design might only imply that active participation in basketball led to an increase in aerobic capacity beside growth and development. In addition to frequency of basketball training (hours/week of training) obtained for the training group, the level and intensity of physical activity could have been measured by accelerometers and heart rate monitors for both evaluated groups in order to get better insight into influence of organized physical activity on monitored parameters.

Conclusion

According to the results presented in the study, maximal oxygen uptake gradually improves with an increase of body size due to growth and development in young girls. It is demonstrated that girls actively engaged in basketball training have higher aerobic capacity and maintain normal values of FAT% and BMI during growth and development. These findings may imply that organized physical activity has a positive influence on evaluated characteristics.

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The significance of the expression of cell proliferation and inflammation markers in the development of acquired middle ear cholesteatoma

Značaj ekspresije markera ćelijske proliferacije i inflamacije u razvoju stečenog holesteatoma srednjeg uva

Milan Erdoglija*, Uglješa Grgurević*, Snežana Cerović†‡, Milena Jović†, Nenad Baletić*‡, Jelena Sotirović*

Military Medical Academy, *Ear, Nose and Throat Clinic, †Institute of Pathology, Belgrade, Serbia; University of Defence, ‡Faculty of Medical Academy, *Ear, Nose and Throat Clinic, †Institute of Pathology, Belgrade, Serbia

Abstract

Background/Aim. Permanent proliferation and periodical infection are the main clinical characteristics of acquired middle ear cholesteatoma. The aim of this study was to research immunohistochemical characteristics of the skin along with the cholesteatoma process in the nearby tissue. This research should influence further studying of etiology and development of acquired middle ear cholesteatoma. **Methods.** We investigated clinical, histological and immunohistochemical characteristics of cholesteatoma in 50 samples from operated patients with acquired middle ear cholesteatomas. We classified all samples according to their clinical characteristics of cholesteatoma such as bone destruction, presence of infection or cholesteatoma extension and histological characteristics of cholesteatoma such as keratinisation, inflammatory process and extracellular matrix proliferation. We used mouse monoclonal antibodies for proliferating cell nuclear antigen (MAbs for PCNA), Ki-67, COX-2, CD 4 and CD 8 lymphocytes to investigate the expression of those characteristics in the cholesteatoma and in the control skin tissue. Statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA). We used the independent group *t*-test, Spearman's correlation analysis and Mann-Whitney U test to analyze statistical analysis. **Results.** Expression of PCNA, Ki-67, COX-2 and CD 8 lymphocytes in more serious clinical picture of cholesteatoma was almost equal as in less serious clinical picture of cholesteatoma. There was statistically significantly higher concentration of inflammation marker CD 4 lymphocytes, both in the acquired cholesteatoma and in the skin of bony portion of the external auditory canal near fibrocartilaginous annulus in more serious clinical picture of cholesteatoma than in less serious clinical picture of cholesteatoma ($p < 0.01$). There was statistically significant difference of expression of PCNA, Ki-67, COX-2, CD 4 and CD 8 lymphocytes between all cholesteatoma samples and the skin of bony portion of the external auditory canal ($p < 0.05$) and statistically significant difference of expression of those markers between the skin of bony portion of the external auditory canal and retroauricular skin ($p < 0.05$). **Conclusion.** Inflammation of the skin of bony portion of the external auditory canal is a milestone in pathogenesis of acquired middle ear cholesteatoma. Expression of CD 4 lymphocytes can be the prognostic factor for acquired cholesteatoma clinical picture development. We found so much diversity in biological behavior through very different levels of cholesteatoma development. Expression of Ki-67 in acquired middle ear cholesteatoma is a reliable and stable marker of proliferation for acquired middle ear cholesteatoma.

Key words:
cholesteatoma; histology; immunohistochemistry; biological markers.

Apstrakt

Uvod/Cilj. Konstantna proliferacija i periodične infekcije su glavne kliničke karakteristike stečenog holesteatoma srednjeg uva. Cilj ove studije bio je da se istraže imunohistohemijske karakteristike kože i holesteatomskog procesa u obližnjem tkivu i tako prostudira etiologija i razvoj stečenog holesteatoma srednjeg uva. **Metode.** Istraživali smo kliničke, histološke i imunohistohemijske karakteristike holesteatoma 50 operisanih bolesnika sa stečenim holesteatomom srednjeg uva. Klasifikovali smo sve uzorke prema kliničkim karakteristikama holesteatoma kao što su de-

Correspondence to: Jelena Sotirović, ENT Clinic, Military Medical Academy, Crnotravska17, 11 000 Belgrade, Serbia.
E-mail: j.sotirovic@gmail.com

strukcija kosti, prisustvo infekcije ili proširenost holesteatomskog procesa i histološkim karakteristikama holesteatoma kao što su keratinizacija, inflamatorni proces i ekstraćelijska proliferacija matriksa. Koristili smo mišija monoklonska antitela za proliferišući ćelijski nuklearni antigen (MAbs za PCNA), Ki-67, COX-2, CD 4 i CD 8 limfocite da bi istražili ekspresiju ovih karakteristika u holesteatomu i u kontrolnom tkivu kože. Statističke analize izvedene su korišćenjem SPSS za Windows, verzija 16.0 (SPSS, Chicago, IL, USA). Koristili smo *t*-test za nezavisne grupe, Spirmanovu analizu korelacije i Mann-Whitney U test za statističku analizu. **Rezultati.** Ekspresija PCNA, Ki-67, COX-2 i CD 8 limfocita u holesteatomima sa težom kliničkom slikom bila je podjednaka kao u holesteatomima sa lakšom kliničkom slikom. Postojala je statistički značajno viša koncentracija CD4 limfocita u uzorcima stečenog holesteatoma i u uzorcima kože koštanog dela spoljnog slušnog hodnika, u blizini fibrokartilaginoznog anulusa, u holestetomima sa težom kliničkom slikom nego u holesteatomima sa lakšom kliničkom slikom ($p < 0,05$). Postojala je statistički značajna razlika ekspresije PCNA, Ki-67, COX-2, CD-4 i CD 8 limfocita izmedu uzorka holesteatoma i uzoraka kože koštanog dela spoljnog slušnog kanala svih operisanih bolesnika ($p < 0,05$) i statistički značajna razlika u ekspresiji PCNA, Ki-67, COX-2, CD-4 i CD 8 limfocita u koži koštanog dela spoljnog slušnog hodnika u odnosu na retroaurikularnu kožu ($p < 0,05$). **Zaključak.** Inflamacija kože koštanog dela spoljnog slušnog hodnika je prekretnica u patogenezi stečenog holesteatoma srednjeg uva. Ekspresija CD4 limfocita može biti prognostički faktor u razvoju kliničke slike stečenog holesteatoma. Našli smo toliko različitosti u biološkom ponašanju holesteatoma na različitim nivoima kliničkog razvoja. Ekspresija Ki-67 u stečenom holesteatomu srednjeg uva je pouzdan i stabilan marker proliferacije stečenog holesteatoma.

Ključne reči:
holesteatom; histologija; imunohistohemija; biološki pokazatelji.

Introduction

Cholesteatoma represents cystic, benign, pseudotumor lump (sac), limited with keratinized squamous epithelium with stroma of granulation tissue, which occurs in various places in body, including within the middle ear. In 1964 Gray [1] described cholesteatoma as “skin in the wrong place”, without hair and glands, while Sade [2] defined cholesteatoma as "the existence of the squamous epithelium of the tympanic cavity, which produces macroscopic amounts of keratin". Cholesteatoma is a chronic disease of the middle ear which resorbs bone [2]. Cholesteatoma can damage hearing and vestibular function and sometimes leads to exocranial and endocranial life-threatening complications. Not a single theory has been able to explain the clinical characteristics of acquired cholesteatoma: uncoordinated proliferation, invasion, migration, altered differentiation, aggressiveness and recidivism [3]. Permanent proliferation and periodical infection are the main clinical characteristics of acquired middle ear cholesteatoma. The aim of this study was to research immunohistochemical characteristics of the skin along with cholesteatoma processes in the nearby tissue significant for etiology and development of acquired middle ear cholesteatoma.

By expression of cytokeratin, cytokines and proliferation markers such as proliferating cell nuclear antigen (PCNA) and Ki-67, epithelial proliferation of the cholesteatoma may be explored. PCNA is used to analyze the synthesis of deoxyribonucleic acid (DNA) during the S phase of the cell cycle, which is related to DNA replication, repair, and cellular apoptosis. PCNA is therefore a non-specific indicator of proliferation of the epithelium. PCNA expression is increased in chronic inflammatory processes. Thus PCNA is a marker suitable for the proliferation assay of cholesteatoma matrix, where the expression of PCNA is higher than in the epidermis. Ki-67 nuclear antigen is a marker of cell proliferation which is expressed in all active phases of the cell cycle (G1, S, G2 and M), while it is not expressed in the inactive phase of the cell cycle (G0). It is assumed that Ki-67 is responsible for chromosome coupling and decongestion, chromosome stabilization and protection and regulation of the symmetric distribution of the nuclear proteins in cell division [4]. Depending on the presence of Ki-67 in the nucleus, in the nucleolus or in the nucleoplasm, expression of the Ki-67 is determined as a granular, diffuse or mixed cell type [5]. Ki-67 has a specific immunoreactivity: it is detectable in the basal and suprabasal layers of the acquired cholesteatoma matrix, while Ki-67 appears only in the basal layer of the epithelium of normal skin [6]. Ki-67 and PCNA are particularly concentrated in the skin near tympanic annulus, where it takes the clip skin of external auditory canal suitable for histopathologic and immunohistochemical analyses. COX-2 (cyclooxygenase 2) is an enzyme generated by decoding PTGS2 (prostaglandin-endoperoxydsynthetize 2) gene. COX-2 is involved in conversion of arachidonic acid to prostaglandin H2 – the precursor of prostacyclin and thromboxane A2, the mediators of the inflammatory process [7]. COX-2 is inactive in healthy cells. The activation of COX-2 occurs in proliferating cells during the development of inflammatory or tumor processes in the body. COX-2 occurs as a response to extracellular stimulation, proinflammatory cytokines or cell growth factors [8, 9].

By expression of immune cells markers, such as CD4 and CD8 T cells in the cholesteatoma, as well as Th1 and Th2 helper immune cells, immune process in the cholesteatoma development can be followed. CD4 and CD8 T lymphocytes are the effector cells which act as cytotoxic or immune cells in the humoral immune response in the cholesteatoma development, after their maturation in the lymph nodes and their migration to the site of infection [10].

Methods

The research was designed as cross-sectional, study. The study included 50 patients between 10–77 years of age,

both sexes (34 male, 16 female) who were operated with the diagnosis of acquired middle ear cholesteatoma. The study was conducted from 2012–2015 at the Ear, Nose and Throat (ENT) Clinic, Military Medical Academy (MMA) in Belgrade and the Institute of Pathology, MMA in Belgrade, according to the provisions of the Declaration of Helsinki. Subject got the approval from the Ethics Committee of Military Medical Academy on April 1, 2012 according to the order of the Head of the Academy (classified) 3232-1. The main criterion for inclusion of the patients in the study was the diagnosis of acquired middle ear cholesteatoma (Figure 1).

Criteria for the exclusion from the study were associated diseases with the acquired middle ear cholesteatoma, such as malignant neoplasm whichever origin it has and chronic diseases of the skin of the external auditory canal and / or auricular regions as eczema, psoriasis, lupus, etc.

We performed tympanoplasty in all pediatric patients (range 10–19 years old) and in most adults (42 patients score). We performed radical tympanomastoidectomy in 8 adults, reoperations of recurrent cholesteatoma in 6 and operations in 2, because of diffuse cholesteatoma (Table 1). We used high resolution temporal bone computed tomography (CT) to establish the diagnosis of recurrent cholesteatoma before reoperation. Nowadays, improvements in magnetic resonance imaging (MRI) techniques led to getting more accurate details of the cholesteatoma using delayed contrast enhanced T1 weighted imaging and diffusion-weighted imaging [11].

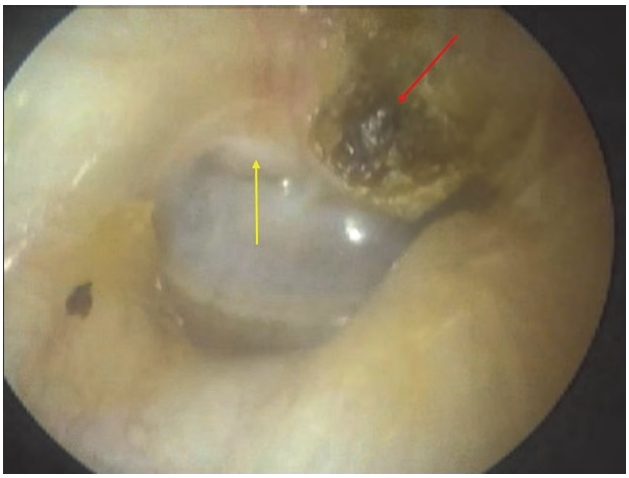

Fig. 1 – Otomicroscopic view of attic cholesteatoma. Cholesteatoma crust (red arrow). Spatium Von Troeltsch - filled with spread attic cholesteatoma (yellow arrow).

Table 1

Distribution of patients according to cholesteatoma clinical classification and type of surgery

Acquired cholesteatoma localization	Number of patients	Tympanoplasty	Radical tympanoplasty
Attic cholesteatoma	21	21	0
Pars tensa cholesteatoma	17	17	0
Diffuse cholesteatoma	3	1	2
Recidivism	9	3	6
Number of operations	50	42	8

The cases were categorized according to the degree of bone destruction (29 patients with more and 21 patients with less bone destruction), the presence of infection (36 patients with and 14 patients without infection) and cholesteatoma extension (34 patients with more and 16 patients with less cholesteatoma extension). We also investigated the correlation of the expression of PCNA, Ki-67, COX-2, CD 4 and CD 8 lymphocytes and the histological characteristics of the cholesteatoma to determine the possible presence of any differences in expression of proliferation and inflammation cell markers according to the degree of keratinization, inflammation or extracellular matrix proliferation. This study also looked at correlation between the immune histochemical and the clinical characteristics of cholesteatoma [12]. Cases were categorized according to clinical findings, i.e. having more bone destruction, cholesteatoma induced erosion of 2 or 3 auditory ossicles, leading to ossicular discontinuity along with erosion of the wall of the middle ear or having less bone destruction with no ossicle damage or one auditory ossicle damage because of the cholesteatoma process. A positive microbial swab before or during surgery indicated the presence of bacterial infection in the cholesteatoma while a negative swab indicated absence of infection. In the group categorized as having more cholesteatoma extension, cholesteatoma was found in all three compartments of the middle ear (i.e. epitympanum, antrum and mastoid cells); in contrast, cholesteatoma extension in one or two compartments of the middle ear defined cases with less cholesteatoma extension (i.e. epitympanum and protympanum).

Samples of cholesteatoma from all 50 cases were soaked in formalin, embedded in paraffin and cut into 4 mm sections using a microtome. All samples were taken from tissue representing complete resection of the cholesteatoma. The slides were deparaffinized, hydrated and washed with TRIS-buffered saline plus 0.5% Tween 20 (TBST buffer). Samples were then subjected to microwave treatment for 30 min in citrate buffer (pH = 6.0) to retrieve the antigens. Endogenous peroxidase activity was blocked using water plus 3% H_20_2 for 30 min. Finally, the cells were characterized using mouse monoclonal antibodies that reacted with the following human proteins: PCNA, Ki-67, COX-2 and CD8 (Serotec); CD4 (Novocastra). Diaminobenzidine was applied for 10 min as a chromogen. The slides were counterstained with Mayer's hematoxylin, washed in water, dehydrated in increasing concentrations of ethanol, cleared in xylene and put in Canada balsam [13, 14].

The number of immune cells that showed positive staining with the cell-specific antibodies was reported semi-quantitatively. Expressions of PCNA, Ki-67, COX-2, CD 4 and CD 8 lymphocytes were scored as follows: 0 – no staning; 1 – 25% positive staining; 2 – 25–50% positive staining; 3 – 50–75% positive staining; 4 – 75–100% positive staining, in one high-powered microscope field (HPF). The histological characteristics of cholesteatomas were scored using a semi-quantitative method to obtain "indexes" for the characteristics. Keratinization was scored as follows: 0 – no keratinization; 1 – low level of keratinization; 2 – moderate level of keratinization; 3 – high level of keratinization. Inflammation/inflammatory cell infiltration was scored as follows: 0 – no inflammatory cell infiltration; 1 – low level of inflammatory cell infiltration i.e. fewer than 5 cells in one HPF; 2 – moderate level of inflammatory cell infiltration, i.e. 5–20 cells in one HPF; 3 – high level of inflammatory cell infiltration, i.e. more than 20 cells in one HPF. Extracellular matrix proliferation (collagen) was scored as follows: 0 – no collagen; 1 – low level of collagen expression; 2 – moderate level of collagen expression; 3 – high level of collagen expression [15, 16].

We used *t*-test for independent groups and the Mann-Whitney U test to statistically analyze the results, obtained. Spearman's correlation analysis was used to test the correlation between two cholesteatoma characteristics. A *p* value less than 0.05 was considered to be statistically significant in all statistic analyses, while a *p* value less than 0.01 was considered to be highly statistically significant.

Results

The mean estimated score of keratinization for all samples was 2.20 ± 0.904, that is a moderate level of keratinization. More than 10 layers of keratinocytes in the cholesteatoma matrix were followed by expressed inflammatory cells infiltration into subepithelial layer of cholesteatoma perimatrix. The mean estimated index of inflammation/inflammatory cell infiltration for all samples was 2.16 ± 1.017, that is a moderate level of inflammation. The perimatrix was dominantly infiltrated by mononuclear cells with diffuse or aggregate distribution. The degree of extracellular matrix proliferation was very different in cholesteatoma perimatrix. The mean estimated index of extracellular matrix proliferation for all samples was 1.92 ± 1.158, that is a moderate level of collagen expression.

We studied and compared the expression of cellular proliferative and inflammatory markers PCNA, Ki-67, COX-2, CD4 and CD8 T cells in 3 tissue preparations of each of the operated patients: a composition of cholesteatoma, a composition of skin of the external auditory canal and preparation of the retroauricular skin regions of the total of 50 treated patients with the acquired middle ear cholesteatoma. Also, we investigated the effect of proliferative and inflammatory parameters in the development of acquired cholesteatoma, comparing the expression of PCNA, Ki-67, COX-2, CD4 and CD8 T lymphocytes in different kinds of cholesteatoma grouped according to histological and clinical characteristics of the acquired cholesteatoma.

PCNA was strongly detectable in cholesteatoma, in relation to the skin of the external auditory canal and to the skin of the retroauricular regions. The expression of PCNA was detected in proliferating cells of the basal layer and suprabasal layer of the cholesteatoma matrix and rarely in the upper layers of the matrix. PCNA expression was detected in the granular layer of the matrix when the proliferation of keratinocytes in the matrix of acquired cholesteatoma was increased, which supports the theory that PCNA is a non-selective marker of cell proliferation. PCNA expression was less in acquired cholesteatoma without infection, but with higher keratin deposits and with increased keratinization. Compared to acquired cholesteatoma, PCNA expression in the skin of the external auditory canal and in the skin of the retroauricular region is less frequently detected, especially in the skin of the retroauricular region, where PCNA staining was mainly detected in individual cells.

Ki-67 showed a great detectability in cholesteatoma, in relation to the skin of the external auditory canal and of the retroauricular region. The increased number of proliferating keratinocytes, which have a specific Ki-67 immunoreactivity, was detected in the basal and suprabasal layer, and less frequently in the upper layers of the matrix of all examined cholesteatoma. Expression of keratinocyte marker Ki-67 was present in all active phases of the cell cycle, which was best illustrated by a different type of nuclear staining (Figure 2).

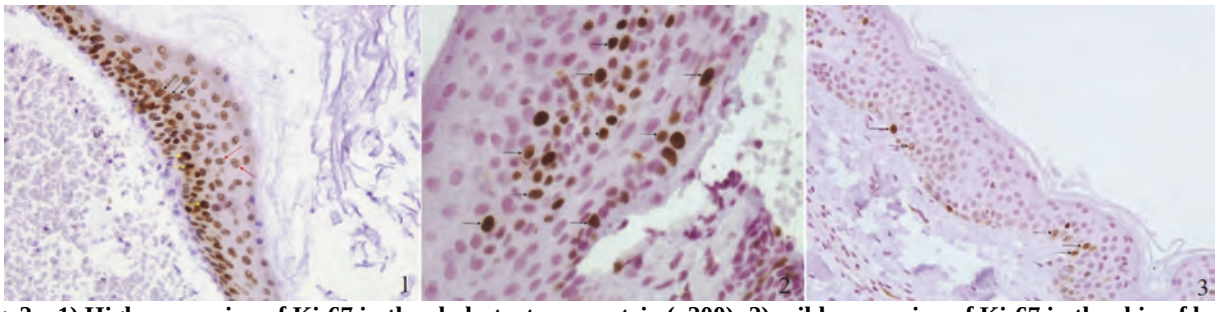

Fig. 2 – 1) High expression of Ki-67 in the cholesteatoma matrix (x200); 2) mild expression of Ki-67 in the skin of bony portion of the external auditory canal (x400); 3) low expression of Ki-67 in the retroauricular skin (x200). Different Ki-67 nuclear staining in keratinocytes: granular type (red arrows), diffuse type (yellow arrows) and mixed type (blue arrows).

COX-2 was detected in all samples of acquired cholesteatoma, mainly as a low to moderate staining expressed (immunoreactivity 50%) of the matrix of cholesteatoma. The estimated expression of COX-2 in the acquired cholesteatoma (immunoreactivity over 50%) was in a small number of cases (18% of total respondents), and followed by prominent lymphocyte proliferation in perimatrix, and increased keratinocytes proliferation in the matrix. Also there was a significant diversity in the expression of COX-2 in the samples of the skin of external auditory canal. COX-2 was almost undetected in the samples of the retroauricular skin.

CD4 marker was perfectly manifested in the subpopulation of helper T lymphocytes in the samples of acquired cholesteatoma. Abundant diffuse infiltrates of CD4 lymphocytes were detected in the perimatrix or there were smaller or larger deposits of cells in the so-called cluster formations. The expression of CD4 cells in the matrix of the cholesteatoma was generally scarce to poorly expressed, while CD4+ cells morphology was diverse, predominantly rounded, occasionally atypical, elongated, or star shaped, with more or less nucleus hyperhromasia.

The expression of CD4 lymphocytes was mostly poor to moderately expressed in the samples of skin of the external auditory canal with a predominance of subepithelial CD4+ cells distribution, while detectability of CD4+ cells in the retroauricular skin was feeble (Figure 3).

The expression of CD8 lymphocytes was lower than the expression of CD4 in the samples of the acquired cholesteatoma with subepithelial cytotoxic T lymphocytes distribution in the cholesteatoma perimatrix. The expression of CD8 lymphocytes in the skin of the external auditory canal was less than the expression of CD8 lymphocytes in acquired cholesteatoma, while expression of CD8 lymphocytes in the retroauricular skin was minimal.

Results of comparing tissue samples of acquired cholestaetoma and skin of the external auditory canal and retroauricular skin according to the clinical, histopathological and immunohistochemical characteristics of acquired cholesteatoma are presented in Tables 2 and 3. There was not astatistically significant difference in index correlation of keratinization, inflammatory infiltration and proliferation of collagen according to the degree of bone destruction of the acquired cholesteatoma.

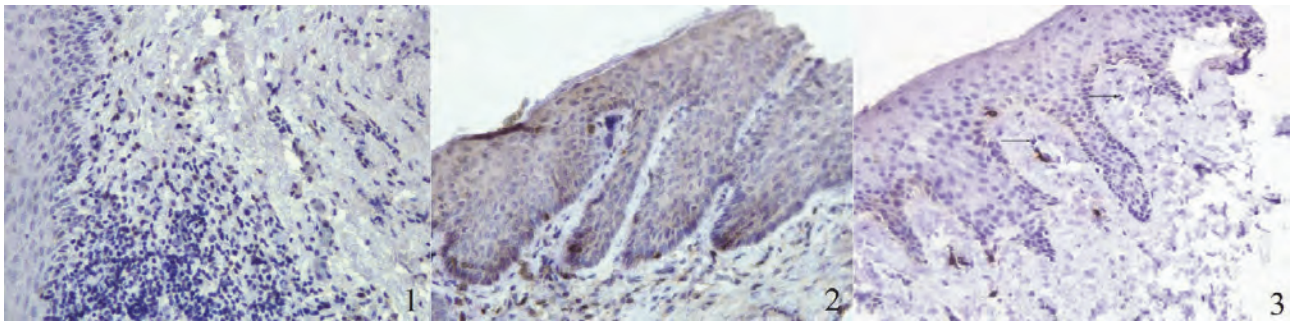

Fig. 3 – 1) Perimatrix cluster infiltration of the acquired cholesteatoma with CD4 lymphocytes and individual CD4 + cells expression in the matrix (x 200); 2) Subepithelial distribution of T lymphocytes expressed with CD4 marker in the skin of the external auditory canal (x200); 3) Individual T lymphocytes (black arrows) expressed with CD4 marker in the retro auricular skin (x200).

Table 2

Histological and immunohistochemical characteristics of cholesteatoma according to degree of bone destruction of the acquired middle ear cholesteatoma

Bone destruction	Keratinization	Inflamm. infiltrate	Collagen	PCNA	Ki-67	COX2	CD4	CD8
More bone destruction	29	29	29	29	29	29	29	29
Index	2.10	2.28	1.97	3.79	3.41	2.39	3.93	3.07
SD	0.976	0.960	1.149	0.412	0.780	0.620	0.408	0.913
Less bone destruction	21	21	21	21	21	21	21	21
Index	2.33	2.00	1.86	3.81	3.00	2.03	3.24	2.45
SD	0.796	1.095	1.195	0.402	0.949	1.076	0.982	1.061
χ^2 test	2.149	2.160	0.144	0.021	3.210	2.278	25.384	1.149
Df	3	3	3	1	3	2	1	2
p	0.542	0.540	0.986	0.886	0.360	0.320	0.001*	0.563

SD – standard deviation; Df – degree of freedom; *p* – probability; *highly statistically significant; PCNA – proliferating cell nuclear antigen; COX2 – cyclooxigenase 2.

Table 3

The expression of cell proliferation and inflammation markers in the skin of external auditory canal and retroauricular skin according to degree of bone destruction of the middle ear cholesteatoma

Bone destruction	PCNA		Ki-67		COX2		CD4		CD8	
	ssh	ra	ssh	ra	ssh	ra	ssh	ra	ssh	ra
More bone destruction	29	29	29	29	29	29	29	29	29	29
Index	2.62	1.69	2.24	1.55	2.32	1.69	3.21	1.71	2.12	1.33
SD	0.622	0.471	0.830	0.506	0.871	0.471	0.904	0.609	0.734	0.453
Less bone destruction	21	21	21	21	21	21	21	21	21	21
Index	2.71	1.71	2.05	1.43	2.12	1.76	2.24	1.66	1.96	1.23
SD	0.463	0.463	0.498	0.507	0.740	0.539	0.692	0.512	0.634	0.412
χ^2 test	1.551	0.035	3.658	0.739	3.353	1.414	18.443	1.409	1.149	1.763
Df	2	1	3	1	2	2	3	1	2	1
p	0.460	0.851	0.301	0.390	0.187	0.493	0.001*	0.235	0.563	0.184

SD – standard deviation; Df – degree of freedom; *p* – probability; *highly statistically significant; ssh – external auditory canal; ra – retroauricular skin; PCNA – proliferating cell nuclear antigen; COX2 – cyclooxygenase 2.

There was a statistically significant difference in the index of keratinization according to the presence/absence of cholesteatoma infection ($p = 0.028$), showing that keratinization was more expressed in cholesteatoma without infection. There was a statistically significant difference in the index of inflammatory infiltration of cholesteatoma ($p = 0.049$) according to absent/present cholesteatoma infection, whereby cholesteatoma with infection tended to show mainly conspicuously marked inflammatory cell infiltration (24 of 36 respondents, or 67% of the patients in the group of cholesteatoma with infection had markedly pronounced inflammatory infiltrate) in the histopathological examination (Figure 4).

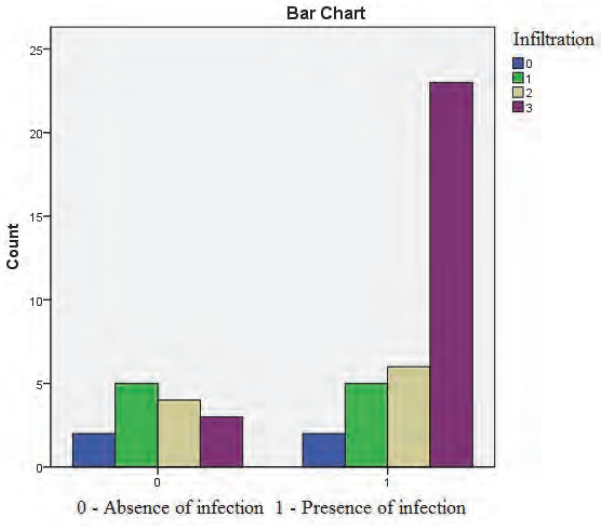

Fig. 4 – Inflammatory infiltrate index in the group of patients with acquired cholesteatoma of the middle ear with absent/ present infection.

0 – no inflammatory cell infiltration; 1 – low level of inflamatory cell infiltration; 2 – moderate level of inflammatory cell infiltration; 3 – high level of inflammatory cell infiltration.

There was no statistically significant difference in the index of histopathologic features of acquired cholesteatoma and the expression of markers PCNA, Ki-67, COX2 and CD8 lymphocytes in cholesteatoma between the groups of patients with more bone destruction and the group of patients with less bone destruction in the middle ear. There was a highly statistically significant difference in the expression of CD4 lymphocytes in the cholesteatoma ($p = 0.001$) and in the expression of CD4 lymphocytes in the skin of the external auditory canal ($p = 0.001$) compared to the level of bone destruction (Tables 2 and 3).

There was a statistically significant difference in the expression of CD4 lymphocytes in cholesteatoma ($p = 0.041$) and in the skin of the external auditory canal ($p = 0.048$) according to the presence/absence of the infection. There was a statistically significant difference in the expression of CD4 lymphocytes in cholesteatoma ($p = 0.007$) and in the expression of CD4 lymphocytes in the skin of the external auditory canal ($p = 0.020$) according to the extensiveness of cholesteatoma. There was a strong CD4 + cell staining in the acquired cholesteatoma and in the skin of the external auditory canal in the cholesteatoma samples with higher extensiveness of the cholesteatoma process.

We used Spearman's correlation coefficient for testing the relationship of the histopathological features in the acquired middle ear cholesteatoma. For histopathological characteristics of cholesteatoma it showed highly statistically significant negative correlation between keretanization and inflammatory infiltration in cholesteatoma ($p = 0.008$), highly statistically significant negative correlation between keratinization and collagen proliferation in cholesteatoma ($p = 0.002$), and a highly statistically significant positive correlation between inflammatory infiltration and collagen proliferation in cholesteatoma ($p = 0.001$). Keratinization in the acquired middle ear cholesteatoma was particularly pronounced in lower inflammatory infiltration and less collagen proliferation. Inflammatory infiltration in the middle ear acquired cholesteatoma was correlated with the collagen development in the perimetrix.

Expression of the markers PCNA, Ki-67, COX-2, CD4 and CD8 T lymphocytes in cholesteatoma according to histopathological characteristics of cholesteatoma showed equal values, with no statistically significant differences.

There was a highly statistically significant difference in expression of all markers PCNA, Ki-67, COX-2, CD4 and

CD8 T lymphocytes in cholesteatoma compared to the expression of the same markers in the skin of the external auditory canal and retroauricular skin. Also the expression of all markers PCNA, Ki-67, COX-2, CD4 and CD8 T lymphocytes in the skin of the external auditory canal was highly statistically different from the expression in the retroauricular skin.

Discussion

Examining the 50 operated patients with acquired middle ear cholesteatoma in our study, we obtained statistically significant higher expression of CD4 T lymphocytes in acquired cholesteatoma with more severe clinical picture, greater bone destruction, present infection as well as increasing extensiveness of the cholesteatoma process. We got a similar result for the expression of CD4 T lymphocytes in the skin of the external auditory canal. CD8 T cells did not show such a distribution in the acquired cholesteatoma and in the skin of the external auditory canal as CD4 T lymphocytes. CD8 T lymphocytes expressed less than CD4 T lymphocytes in cholesteatoma and in surrounding skin. Possibly, CD 8 T lymphocytes cytotoxicity had less importance in the immunological processes in the acquired cholesteatoma. CD 8 T cells had equal distribution and equal activity in different clinical groups of cholesteatoma. The other authors also favored the humoral immune response in the development of acquired cholesteatoma, which was closely linked to the presence of Langerhans cells in acquired cholesteatoma and the increased presence of CD4 T lymphocytes [17, 18]. CD4 T lymphocytes were the main carriers of the inflammatory infiltrate in our study, especially in strikingly expressed inflammatory infiltrates which could be found in 67% of respondents with infection. Lymphocytic infiltrates were predominantly stained in the subepithelial perimatrix in diffuse distribution with the possibility of good communication to the cholesteatoma matrix. Inflammatory infiltration was statistically significantly higher in acquired cholesteatoma with infection in comparison with acquired cholesteatoma without infection, which was evidenced in some earlier articles [15]. Ma et al. [19] showed, by comparing different places for cholesteatoma sampling, that the clinical picture of acquired cholesteatoma went with large inflammatory infiltrate in permatrix. Cholesteatoma cases sampled from purulent, bone destruction parts of the middle ear had the most expressed inflammation [19]. Acquired cholesteatoma developing for years got the aggressive, bone destruction form during the direct contact between cholesteatoma and bones. Immunological events in the inflammatory infiltrate of cholesteatoma perimatrix were responsible for bone destruction of the middle ear. Inflammatory infiltrate of the acquired middle ear cholesteatoma was correlated with the development of collagen in perimetrix in our study. Collagen was produced by fibroblasts in acquired cholesteatoma perimatrix as sequelae in the final stages of the inflammatory process [13]. Keratinisation of the acquired middle ear cholesteatoma was less pronounced due to increased inflammatory infiltrate, increased extracellular matrix, or collagen in our study. More expressed inflammatory process in cholesteatoma perimatrix led to more bone destruction in the middle ear. The phenotypic diversity of cholesteatoma may explain lesser or greater inflammation, but not its origin [19].

Keratinization was negatively correlated with the infection of acquired cholesteatoma. Keratinization was statistically significantly higher in cholesteatoma without infection in comparison with cholesteatoma with infection in our study. Regular bulbous appearance of adherent keratin deposits in the cholesteatoma without infection, with a thin matrix of cholesteatoma is a typical histopathological picture of the slowly growing acquired cholesteatoma without exacerbation of infection. Thin layers of the cholesteatoma matrix with greater keratinization suggest the problem of keratinocyte differentiation in the cholesteatoma development. More keratin accumulation in keratinocytes during corneocytes creation contibutes to a process of separating the lamellar layers and the keratin creating bulb keratin as a standard phenotypic characteristics of these cholesteatoma. The apoptosis of corneocytes which occurs not only in the horny layer of the matrix of the cholesteatoma (at 2–4 splitter), but also in the lower, contibutes to a great amount of amorphous keratin. According to our research, keratinization correlated with abnormal keratinocyte differentiation. There was a poorly expressed inflammatory infiltration in perimatrix of these cholesteatomas, which probably means that there is a poor and paracrine communication between the matrix and the perimatrix. The reason for the accelerated maturation and disturbed differentiation of keratinocytes accompanied by apoptosis of epithelial cells should be seek searched in the intercellular matrix developments or physical factors as a type of pressure, acid-base status, hypoxia, etc. [20]. The expression of cell proliferation markers PCNA and Ki-67 in keratinocytes in our study did not show statistically significant differences according to the clinical and histopathological characteristics of the acquired cholesteatoma. The test results obtained from Ki-67 expression of keratinocyte proliferation in the acquired cholesteatoma in children and adults are controversial. Welkoborsky [9], and Dornelles et al. [13] showed no difference in keratinocyte proliferation obtained from the children and from the adult form of cholesteatoma, while Aslıer et al. [14] showed a statistically greater Ki-67 expression in adults cholesteatoma compared to children cholesteatoma. Li et al. [16] showed significantly higher Ki-67 expression in cholesteatoma with pronounced bone erosion than in cholesteatoma with less bone erosion. Sanli et al. [21] showed no significant differences in Ki-67 expression in primary and recurrent acquired cholesteatoma. The proliferation of keratinocytes was significantly higher in the skin of the external auditory canal than in the retroauricular skin, but also the proliferation of keratinocytes was significantly higher in cholesteatoma matrix than in the skin of the external auditory canal, analyzing the expression of PCNA and Ki-67, which proved to be expected and was in line with the findings of other authors [6, 22, 23].

Clinical verification of acquired cholesteatoma infection was obtained by positive bacteriological swab test. In our study 72% cholesteatoma samples were infected by indi-

vidual or mixed bacterial flora. The infectious agent obtained initiation of cholesteatoma matrix causing additional activation of the keratinocytes proliferation. The infectious agent can be proliferation of the endotoxin from the bacterial cells wall, fragments of the keratin debris, biofilm, etc. Recent studies show a significant role of bacterial biofilms which is identified as the infectious agent and trigger in the development of chronic suppurative otitis with cholesteatoma [24]. The presence of bacterial biofilms from the accumulated keratin may be the evidence of latent cholesteatoma infection in the group of 28% of the patients with ear swab – negative tests, in our study. Bacteria or fungi inside the biofilms were resistant to antibiotics and the immune response of the host and led to a state of chronically infected cholestetaoma [25].

By exploring histopathological features of acquired cholesteatoma it is shown that there is much diversity of cholesteatoma structure, which correlates with the stage of cholesteatoma development. The heterogeneous morphological images of the acquired cholesteatoma do not show precise diversity of the biological behavior of cholesteatoma in different samples. The histopathological findings of the acquired cholesteatoma reflect only one moment in the cholestatoma development. The results of the histological examination should therefore be commented with consideration of the time when the sample tissue was taken and anatomical site from which the sample tissue was taken.

Conclusion

We found much diversity in biological behavior through very different levels of cholesteatoma development. Keratinisation index of acquired middle ear cholesteatoma was higher in the absence of infection. Inflammatory cell infiltrate index of acquired middle ear cholesteatoma was higher in cases with infection. Keratinisation index had negative correlation with inlammatory cell infiltrate index and extracellular matrix proliferation index of acquired middle ear cholesteatoma. Inflammatory infiltrate had positive correlation with colagen development in perimatrix of acquired middle ear cholesteatoma. Inflammation in the skin of bony portion of the external auditory canal is a milestone in etiology and pathogenesis of acquired middle ear cholesteatoma. Expression of Ki-67 in acquired middle ear cholesteatoma is a reliable and stable marker of proliferation for acquired middle ear cholesteatoma. Expression of CD 4 lymphocytes can be a prognostic factor for acquired cholesteatoma clinical picture development.

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GENERAL REVIEW

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Visual evoked potentials – current concepts and future perspectives

Vizuelni evocirani potencijali – sadašnji koncepti i buduće perspektive

Jasna Jančić*, Nikola Ivančević*, Blažo Nikolić*, Mirjana Popović†, Žarko Martinović‡, Dejan Stevanović*, Marina Grbić§, Vesna Djurić‖, Janko Samardžić¶

University of Belgrade, Faculty of Medicine,*Clinic of Neurology and Psychiatry for Children and Youth, ‡Institute for Mental Health, §Institute for Oncology and Radiology, ‖University Children's Hospital „Tiršova“, ¶Institute for Pharmacology, Clinical Pharmacology and Toxicology; Faculty of Electrical Engineering, †Department for Signals and Systems, Belgrade, Serbia

Key words:
evoked potentials, visual; multiple sclerosis; migraine disorders; epilepsy; optic nerve diseases; diagnosis.

Ključne reči:
evocirani potencijali, vizuelni; multipla skleroza; migrena; epilepsija; n. opticus, bolesti; dijagnoza.

Introduction

Sensory evoked potentials (EPs) represent changes in electrical activity of the nervous system, triggered by stimulating sensory receptors or peripheral nerves or either an external or internal impulse. Although every sensory modality can be investigated, sensory EPs mostly used in clinical practice are the following three types: visual evoked potentials (VEP), short latency brainstem auditory evoked potentials (BAEP) and somatosensory evoked potentials (SSEP) [1]. The above EPs modalities are commonly used in combination, as complementary methods in clinical neurophysiology, so they are called multimodal EPs [2]. EPs can also represent brain response as a result of cognitive activity (event related response – ERP) [1].

EPs are recorded in different clinical contexts. They may be used to assess peripheral sensory function, to evaluate the functional integrity of sensory projection pathways in the central nervous system (CNS), and cerebral cortical sensory areas [3].

EPs are recorded by using scalp electrodes for standard electroencephalography (EEG) [1]. Due to low amplitudes of EPs, computer summation or averaging is necessary to isolate them from the background "noise" consisting of spontaneous electrical brain activity on which EPs are superimposed [1,3,4].

EPs were introduced in the early years of clinical EEG within 1930s. The first device for signal processing in the field of EPs using signal averaging method was introduced by Dawson in 1951, while widespread use was enabled in 1970s [4].

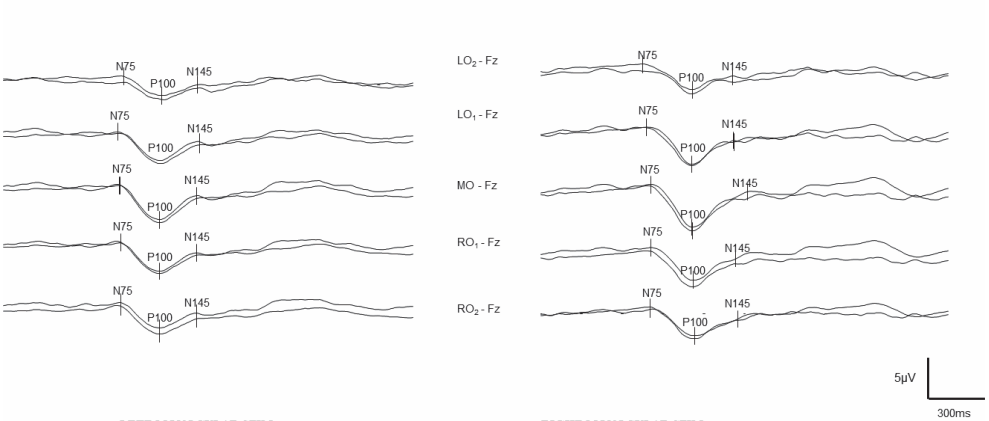
Non-invasiveness and harmlessness both represent the clear advantages of EPs, as well as their repeatability, objectivity and resistance to drugs and anaesthetics. On the other hand, the disadvantage of EPs is their low disease specificity [2,5].

Visual evoked potentials – background

Visual evoked potentials (VEPs) are electrophysiological responses to stimulation by either patterned or un-patterned visual stimuli. Low rate stimulation, referring to pattern checks shifts (reversal of black and white) up to 4 Hz (mostly 1–2 Hz), produces "transient" VEPs. Stimulation at higher rates (≥ 10 Hz) produces responses occurring at the same frequency, lasting during the stimulation as "steady-state" VEPs. Responses evoked by patterned stimuli are "pattern" VEPs or PVEPs whereas those evoked by unpatterned stimuli are "flash" VEPs or FVEPs [1,6].

In healthy individuals, low rate stimulations PVEP have a tendency to produce typical "V" shaped wave (Figure 1). This wave consists of 3 components (often named "picks"), marked as N1 or N75 (referring to mean latency in ms, at which the response will occur after stimulation), P1 or P100 (representing the most important and stable component of the response) and N2 or N145. N and P represent negative and positive deflections in the response wave [1,6].

Correspondence to: Jasna Jančić, University of Belgrade, Faculty of Medicine,Clinic of Neurology and Psychiatry for Children and Youth, Dr Subotića 6a, 11000 Belgrade, Serbia. Phone +381 11 2658 355. E-mail: jasna.jancic.npk@gmail.com



LEFT MONOCULAR STIM.

	N75 ms	P100 ms	N145 ms	P100 μV
1.1 LO_2 – Fz	89	116	143	2.3
1.2 LO_1 – Fz	84	115	144	3.6
1.3 MO – Fz	85	114	142	4.5
1.4 RO_1 – Fz	86	114	142	3.7
1.5 RO_2 – Fz	86	114	142	3.4
2.1 LO_2 – Fz	88	116	142	2.6
2.2 LO_1 – Fz	84	115	144	4.2
2.3 M0 – Fz	86	114	143	4.9
2.4 RO_1 – Fz	86	114	142	4.1
2.5 RO_2 – Fz	86	114	142	3.6

RIGHT MONOCULAR STIM.

N75 ms	P100 ms	N145 ms	P100 μV
78	110	142	3
78	111	142	4.9
81	111	152	5.6
81	112	143	4.2
81	113	145	3.5
75	112	141	3.4
76	111	142	5
79	112	150	5.9
79	112	144	4.7
81	113	144	4.2

Fig. 1 – Normal full-field pattern visual evoked potentials (PVEP) finding in a female subject aged 51. Recording montages the Queen Square System of electrode placement (MO: midoccipital, in midline 5 cm above inion; LO_1, RO_1, LO_2 and RO_2, lateral occipital, 2.5 and 5 cm to left and right of MO, respectively). Each trace represents different electrode placement with the same stimulation pattern (monocular stimulation).). Two responses were recorded to ensure reproducibility of major response components.

The clinical interpretation of PVEP is mostly based upon the latency of P100 and to a much lesser extent to its P100 amplitude [7]. In clinical analysis of multifocal visual evoked potentials (mfVEP), the magnitude (amplitude) of responses and inter-ocular differences are often more relevant finding than latency delay [8].

FVEPs are less sensitive than PVEPs. Therefore, their use in clinical testing is limited to subjects who cannot visually resolve a pattern stimulus due to severe refractive errors or the opacity of ocular media and to those who are too young or not cooperative enough to be able to fixate reliably on a pattern stimulus [1, 6, 7]. After flash stimulation, FVEPs typically consist of up to six peaks in the first 250 ms, labelled sequentially from I to VI. The latency of the individual peaks may show considerable variations among the patients. For this reason, their clinical relevance is reduced with the absence of a demonstrable response being the only definite significant abnormality [1, 6]. This test tends to offer more qualitative than quantitative information [7].

Neuronal generators of VEP are located in the peristriate and striate occipital cortex [6, 7, 9, 10].

Recording techniques and technical aspects

Standard EEG electrodes are commonly used for VEP recording. Electrode placement can be performed by using two internationally approved systems: Queen Square System of placement (occipital leads are labelled LO, MO, and RO) and the International 10–20 System of placement (leads O1, Oz, and O2) [6]. Type of the stimulus, stimulation characteristics and testing protocols depend on the type of VEP being tested [1, 6].

Pattern VEP

Depending on the part of visual field tested, full visual field pattern VEP, partial visual field pattern VEP and multifocal VEP can be defined.

Full visual field pattern VEP can be used for testing lesions of visual system anterior to optic chiasm. This technique is more sensitive for the lesions affecting the central 8–10 degrees of visual field [1, 6].

Lesions affecting half or a part of visual field but sparing the central part are better assessed with partial visual field pattern VEP. This method can detect partial prechiasmal, postchiasmal or chiasmal lesions at the cost of being more time consuming [1, 6].

Computer screen is most commonly used for the presentation of patterns. There are different pattern types, checkerboard patterns being the most extensively studied and used in clinical testing; bar and sinusoidal grating stimuli also produce clinically useful response. Check size is measured using the visual angle (distance from subject eyes to screen should not be less than 70 cm). A fixation point is used as an object to focus the subject's attention. Pattern check reversal rate is less than 4 Hz, usually 1–2 Hz [1, 6].

Multifocal VEP

The multifocal VEP (mfVEP) was introduced in 1994 by Baseler et al. [11]. It is a mathematically improved technique for the extraction of hundreds of VEPs, with the help of only 4 occipital scalp electrodes [4]. This technique uses a multifocal circular dartboard array that usually has two binary m-sequences, each mathematically independent, determining two stimulus states, e.g. two contrast polarities of the pattern [4, 12]. The response is evoked by the change between the two states of the pattern and the stimulation procedure requires 7 to 8 minutes duration for one monocular recording [11–13]. The mfVEP enables separate stimulation of 60 different sectors of full visual field, involving both central and peripheral locations [4, 13]. In this way, standard mfVEP provides a cleaner separation of focal response contributions and is distinct from full-field pattern VEP, which is mostly dominated by responses from macular area [14]. Thus, the main advantage of mfVEP is to demonstrate the topography of visual fields damage with a greater precision than other VEP methods and thus detect localized damage in the form of small scotomata or peripheral visual fields defects [15]. The main indications for mfVEP in ophthalmology include: ruling out functional causes, evaluating patients with unreliable or questionable subjective perimetry tests, and following disease progression [15].

Flash VEP

Unpatterned visual stimuli consist of brief flashes of light with no observable pattern or contour. Stimulation may be presented by a photostimulation lamp (stroboscope), a matrix of light emitting diodes (LEDs, within board or goggles), or a Ganzfeld stimulator. The rate should be approximately 1/s or slower [6].

Influence of subject/patient factors

Age

By the age of 6 to 12 months FVEPs show significant maturational changes; after this period latencies decrease, waveforms merge and FVEPs reach adult morphology [7]. Defining the physiological age in infants is rather difficult, since the nervous system neither matures at the constant rate nor follows the precise defined time table. For this reason it is rather hard to define the precise normative data for an early age of life [16]. During the first 4 to 5 years of life, morphology and latencies of PVEP change as a result of the visual system development. By the age of 5, PVEP resembles that of the adults [7]. Studies have revealed that PVEP P100 latencies tend to increase after the 6th decade, but this increment depends on the check size used in the study. Data for P100 amplitude changes after the 6th decade are scarce [7].

Gender

Females usually have shorter P100 latencies than males [7].

Visual acuity

Generally, visual acuity should be determined before testing VEP [1, 6]. PVEP P100 amplitude is more sensitive to visual acuity changes than P100 latency [7].

Reproducibility

Unlike FVEP, PVEP is very sensitive to the state of the subject's arousal, concentration and attention [1, 6, 7].

Clinical application

Multiple sclerosis and optic neuritis

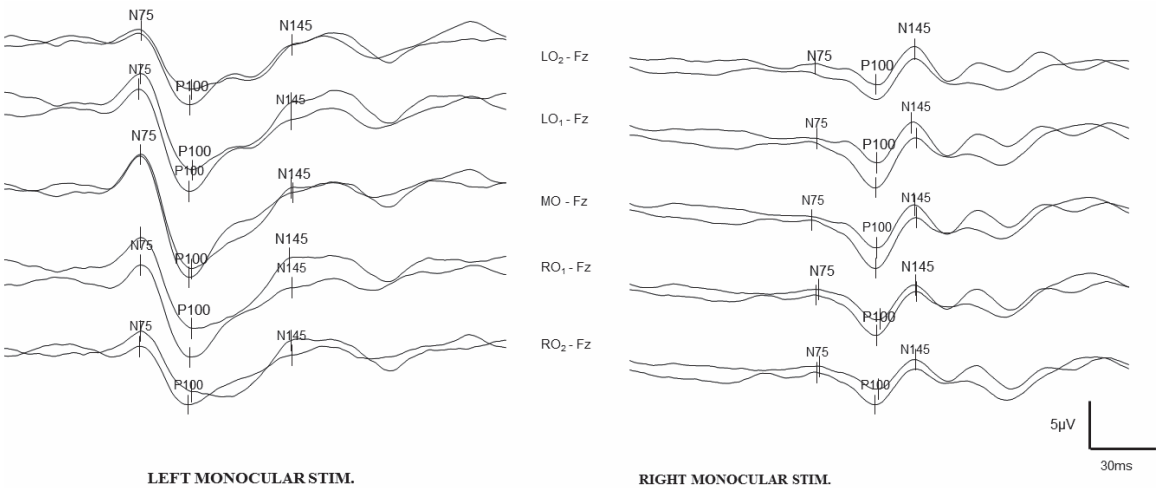
Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the CNS, affecting myelinated axons [17]. Optic neuritis (retrobulbar neuritis) is one of the common disease manifestations [18]. PVEP shows great sensitivity in patients with optic neuritis, having prolonged latencies of P100 wave component in almost all affected subjects (Figure 2). Prolonged P100 latencies were also discovered in more than half subjects having only clinical spinal cord involvement [19, 20]. Compared to SSEP and BAEP, VEPs are the most efficient in detecting the silent lesions in MS [21]. Earlier diagnostic criteria for MS included VEP tests, but due to magnetic resonance imaging (MR) superiority VEPs were later excluded, but are still frequently used [22, 23]. However, new MS diagnostic criteria revision (for 2016) proposes to reintroduce optic nerve lesions as a part of criteria for dissemination in space, suggesting VEP as a useful diagnostic method [24].

Prolonged latencies and reduced amplitudes of VEP can also be found in optic neuritis of different etiology, such as in neuromyelitis optica (NMO). Delayed P100 latencies in the eyes without prior optic neuritis suggest subclinical affection [25]. The mfVEP has the advantage over both the PVEP and perimetry in the follow-up of patients with optic neuritis. Patients converting to clinically definite MS during one year follow-up demonstrate the largest amplitude reduction and the longest latency delay of the optic neuritis eye [26].

Two studies comparing the sensitivity of PVEP and mfVEP in the assessment of patients with optic neuritis caused by multiple sclerosis in 26 [14] and 19 patients [27], respectively. Both studies suggested that the mfVEP have superior performance but in the study that tested the reproducibility, PVEP had also very good sensitivity [27]. Therefore, it was recommended that PVEP, as a more readily available and currently a shorter test, should be used to screen patients for optic neuritis/MS while mfVEP testing has to be added when the PVEP test is negative and the damage is local [27].

Effects of treatment on optic neuritis have been tested with PVEP in many studies [28–32].

Corticosteroids as common medication used for optic neuritis of different etiology can influence VEP latencies.



LEFT MONOCULAR STIM.

	N75	P100	N145	P100
	ms	ms	ms	μV
1.1 LO_2 – Fz	82	112	172	6.4
1.2 LO_1 – Fz	82	113	171	10.4
1.3 MO – Fz	82	112	171	12.4
1.4 RO_1 – Fz	82	112	171	9.8
1.5 RO_2 – Fz	82	112	172	6.5
2.1 LO_2 – Fz	80	111	174	7.7
2.2 LO_1 – Fz	80	111	172	11.0
2.3 M0 – Fz	81	111	173	13.1
2.4 $R0_1$ – Fz	82	111	172	10.0
2.5 $R0_2$ – Fz	81	110	172	6.4

RIGHT MONOCULAR STIM.

N75	P100	N145	P100
ms	ms	ms	μV
112	148	170	2.2
112	148	170	2.6
113	149	171	3.2
114	151	172	3.3
114	149	172	2.5
112	148	171	2.7
112	148	172	4.8
109	148	172	5.2
112	148	172	4.4
112	147	172	3.6

Fig. 2 – Pattern visual evoked potentials (PVEP) finding in male patient aged 27, with optic neuritis as the initial manifestation of multiple sclerosis. Otherwise, the conventions and arrangements were the same as those shown in Figure 1.

The oral methylprednisolone can influence faster improvement of VEP latencies in initial period after optic neuritis onset (up to 4 weeks). In later follow-up (12 weeks and 1 year after onset) there were no benefits of steroid therapy [28].

VEPs, combined with other EPs, proved useful in evaluating the efficacy of drugs designed to impede the course of MS, such as interferon 1b [29], natalizumab [30], and fingolimod [31]. Compared to the pre-treatment delays, latency of PVEPs in these studies improved after the treatment with natalizumab, and VEP sum score was stable in 95% of patients and 5% worsened 1 year after the start of fingolimod treatment [31]. The improvement is most likely explained by the occurrence of remyelination in treated patients (Figure 2) [32].

Migraine

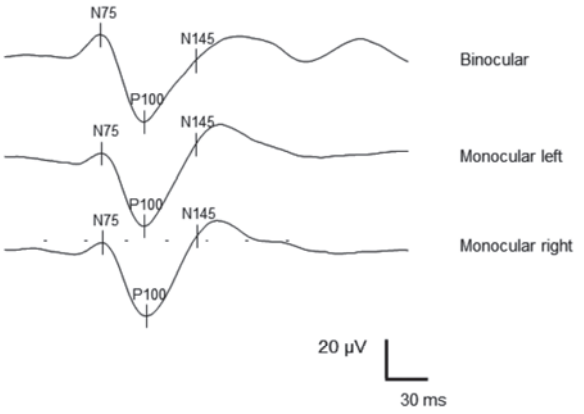
Migraine is considered to be a neurovascular disorder [33]. It is also listed as the sixth highest specific cause of disability in adults [34]. Worldwide prevalence of migraine in children and adolescents was estimated to be between 7% and 11% [35]. Earlier studies have revealed central stimulus processing defects in people with migraine (with and without aura), manifesting as an interictal lack of habituation for acoustic, somatosensory, nocioceptive and visual stimuli [36]. However, the latest research casts a doubt on this finding concerning the lack of habituation measured by PVEP in migraine, considering it as a researcher's bias [37]. Diagnosis of migraine remains predominantly the clinical one, but VEP could be useful as a secondary diagnostic tool. PVEP amplitudes between N1 - P1 and P1 - N2 are significantly larger in children with migraine headaches (Figure 3) [38]. Migraine subtypes in teenage population may also be differentiated on the basis of N2 wave latency prolongation [39].

Neuropathy of optic nerve

Retinal and optic nerve neuropathies of different origin can also influence VEP testing results, affecting both wave latencies and amplitudes [7, 16].

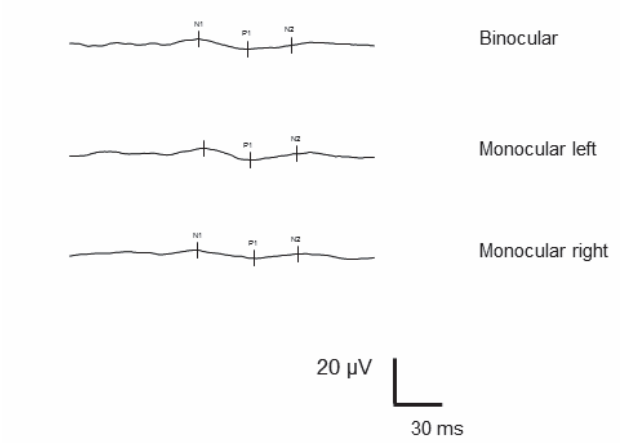
Leber's hereditary optic neuropathy

Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disorder. It is characterized by acute or subacute painless loss of central vision, usually in young adult males [40–42]. PVEP findings are distorted to a great extent, with increased P100 latencies as well as decreased amplitudes. As the disease progresses and the vision fades, only FVEP can be applied showing further prolongation of latencies and the decline of response wave amplitudes (Figure 4) [2, 7, 40]. Multifocal VEP identifies abnormal neural conduction along the visual pathways in LHON, pointing out the involvement of axons driving responses from the central retina [43].



	N75	P100	N145	P100
	ms	ms	ms	μV
1.1 Binocular	71	104	142	43,2
1.2 Mono left	72	103	142	36,1
1.3 Mono right	72	105	143	36,6

Fig. 3 – Pattern visual evoked potentials (PVEP) finding in a female subject aged 11, with migraine headache showing normal latencies and larger amplitudes of evoked response. Recording montage is the International 10-20 System placement (Oz-Fz). Note that each trace represents the same electrode placement, but different mode of stimulation (mono- vs. binocular stimulation).



	N1	P1	N2	N1P1
	ms	ms	ms	μV
1.1 Binocular	127	175	218	7,5
1.2 Mono left	131	177	223	8,3
1.3 Mono right	125	181	224	6,4

Fig. 4 – Flash visual evoked patentials (FVEP) finding in a male subject aged 14, with Leber's hereditary optic neuropathy, showing serious abnormalities with increased latencies and decreased amplitudes. Responses are recorded after light emitting diode (LED) Goggle stimulation.

Glaucoma

Glaucomas are a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells, leading to a characteristic appearance of the optic disc as well as the visual loss [44]. Multifocal VEP is an effective method for detecting visual field loss in glaucoma and represent additional test to subjective automated static perimetry [45]. A comparative study of 50 patients with glaucoma proved that misses and false-positive results occurred with both the automated static perimetry and mfVEP [46]. Therefore, combined use of the two tests may increase the yield of true-positive results indicating glaucomatous damage of ganglion cells.

Ischemic optic neuropathy

Apart from optic neuritis, the most common optic nerve pathology is ischemic optic neuropathy. VEP amplitude decreases significantly in ischemic optic neuropathies, whereas latency delay is more significant in the patients with optic neuritis [47].

Idiopathic intracranial hypertension

Idiopathic intracranial hypertension (pseudotumor cerebri) is a disorder followed by an increased intracranial pressure with no clinical, laboratory or radiological evidence of an intracranial space-occupying lesion.

Prolongation of VEP latencies is observed prior to clinical visual impairment [48]. Repeated VEP showing prolonged latencies in patients with relatively rapid progression of substantial visual field defects may have some prognostic value indicating a need for decompressive neurosurgical treatment to prevent optic atrophy and vision loss [49].

Compressive lesions of the anterior visual pathways

Papilledema arising from the lesions which don't involve optic nerve will not produce P100 alterations unless they are severe. On the other hand, extrinsic and intrinsic tumours compressing anterior visual pathways tend to decrease amplitude and to increase latency of PVEP waveforms [7]. During surgical removal of the tumours which compress anterior visual pathways (e.g. pituitary region tumours), VEP monitoring can be useful. Changes in the latency of P100 and/or changes in the amplitudes of N1-P1 can indicate iatrogenic injury of the visual pathways during an operative procedure [16].

Epilepsy and anti-epileptic drugs (AED)

Epilepsy is very common in childhood. It is estimated that 0.5%–1.0% of all children suffer from epilepsy. The abnormalities of VEPs in epilepsy may be related to the disease itself (seizure types and aetiology) or to the effects of AEDs on the GABA-ergic neurotransmitter system and/or other

CNS functions. Children treated with sodium valproate and carbamazepine have prolonged latencies and reduced amplitudes of P100 wave component of PVEP [16, 50]. The use of VEP and electroretinography (ERG) in children taking vigabatrin may detect visual field constrictions in the early treatment phase and its persistence long time after the drug withdrawal [10, 51].

Conversion disorder

VEPs are commonly used in both adult and paediatric population in order to objectively predict visual acuity in the patients with functional visual loss [16].

VEP in paediatrics

In addition to the aforesaid entities which are also encountered in the paediatric population, VEPs are used in assessment of many disorders specific for childhood: neonatal asphyxia, neurofibromatosis type I (NF1), leukodystrophies, neuronal ceroid lipofuscinosis, coma, hydrocephalus, developmental defects and delay, detection of amblyopia, numerous metabolic and toxic disorders [2, 7, 16, 52].

Combination of VEPs and other neurophysiological methods proved useful in the prognostic assessment of comatose patients and in neurometabolic disorders affecting various levels of CNS. Simultaneous assessment of ERG, VEPs and EEG is useful in the early detection of visual dysfunctions in neuronal ceroid lipofuscinosis (NCL) – the most common neurodegenerative disorder occurring in children. The main use of ERG is in the early diagnosis of juvenile form of NCL [53].

EPs vs. MRI

In comparison with MRI, VEP was far more useful in detecting optic nerve lesions in MS [54] or equally sensitive in detecting subclinical lesions [55]. Nowadays, combined use of gadolinium enhanced MRI and PVEP is very suitable to detect whole brain demyelination and axonal degeneration in MS [56]. SSEP was less sensitive than MRI in detecting spinal cord lesions. BAEP was able to localize lesions along the auditory pathways at a rate which was almost similar to that of MRI. EPs can be used when MRI is negative or cannot be performed. They can also be performed in treatment response evaluation, long-term prognosis and nonspecific changes on MRI [57].

New tendencies in the VEP application

Combined use of MRI 3Tesla scanner and mfVEP technique in the follow-up of 30 patients with acute optic neuritis demonstrated that lesion length and mfVEP latency and were strongly correlated [58]. Future studies of this type may give new insight into the structure-function relationships during optic nerve demyelination and remyelination processes, and axonal degeneration.

Some new technical systems apply VEP in Brain–computer interface (BCI) paradigms to help people with motion disability. For example, steady-state visual evoked potentials (SSVEPs) are frequently used as a control signals as they can offer the user to select among several commands, suitable to drive a BCI based menus. Each option/command in such menu is associated with one of the stimuli presented to the user, differing from each other only by their repetition frequency. All stimuli are simultaneously presented and the user can choose one by focusing the visual attention to it, eliciting the corresponding SSVEP response in the EEG measured over the primary visual cortex. The SSVEP amplitude is greater for the attended stimuli than for the unattended ones, even when the stimuli are presented in the same region of visual field. These SSVEP based BCIs are developed for communication and/or control of electrical devices for different purposes (for example, a wheelchair) [59, 60]. Recent findings show the potential of BCI technology to be used either for long term substitution or further enhancement of the impaired motor function, defining two approaches in BCI applications for neurorehabilitation: assistive and restorative, respectively (for example, SSVEP-based selection of the appropriate electrical stimulation pattern for intended type of trained grasp) [61].

Late wave component of VEP, named P300 (P300 event related potential) is regarded as a neurophysiologic indicator of cognitive processing of a stimulus. This response can also be induced by using the auditory or somatosensory stimulus, and it is usually detected between 300 and 600 ms after stimulus presentation. It is widely used in the field of cognitive neuroscience [16, 62].

Conclusion

Visual evoked potentials are very important additional clinical method in diagnosing of many diseases in neurology, as well as their follow-up. Owing to their non-invasiveness, simplicity of implementation, repeatability, low cost and reliability, VEPs are widely used in many research areas of neuroscience. A special advantage of VEPs is their application in low compliance subjects, especially young children and comatose patients. With the advancement of computed technology and neurophysiology, the possibilities of VEP applications have become reality.

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CASE REPORT

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Orthodontic treatment of a severe unilateral open bite and crossbite, by palatal appliance with monolateral screw (by Veltri). A case report

Ortodontska terapija izraženog unilateralno otvorenog i ukrštenog zagrižaja, pomoću palatinalnog aparata sa monolateralnim šrafom (Veltri)

Tatjana Perović*†, Ilija Aleksić†, Zorica Blažej‡

University of Niš, *Faculty of Medicine, Niš, Serbia; †Independent Odontotechnician, Niš, Serbia; Dental Clinic, ‡Department for Orthodontics, Niš, Serbia

Abstract

Introduction. An appliance according to Veltri is a kind of palatal construction which is rarely used in our region. It appeared as a logical consequence of the evolution of appliances for rapid palatal separation. It is primarily indicated for upper molars distalization. However, its good qualities allow the use it in case of some other orthodontic problems. **Case report.** The aim of this report is overview of the therapy of 14-year-old boy with asymmetry of the upper dental arch and unilateral open bite and crossbite, using the appliance according to Veltri. The plan of therapy was primarily aimed at correction of upper arch asymmetry by using appliance according to Veltri. Extraction of the lower first premolars was done and then upper and lower fixed appliance for leveling the upper and lower dental arch, normalization overjet and the depth of overlap was placed. The total duration of the therapy was two years. **Conclusion.** Presented combined camouflage therapy achieved a satisfactory result, regardless of the poor prognosis due to hyperdivergent growth.

Key words:
malocclusion; orthodontic appliance design; cephalometry; dental arch; treatment outcome.

Apstrakt

Uvod. Aparat po Veltriju predstavlja vrstu palatinalne konstrukcije, koja se kod nas retko koristi. Aparat je nastao kao logična posledica evolucije aparata za rapidnu ekspanziju palatuma. Indikovan je, pre svega, za distalizaciju gornjih molara. Međutim, njegove dobre osobine, omogućavaju njegovu upotrebu i u terapiji drugih ortodontskih problema. **Prikaz bolesnika.** Cilj prikaza je terapija četrnaestogodišnjeg dečaka sa asimetrijom gornjeg zubnog luka i unilateralno otvorenim i ukrštenim zagrižajem pomoću aparata po Veltriju. Plan terapije je predviđao, primarno, korekciju asimetrije gornjeg zubnog luka aparatom po Veltriju. Zatim je sledila ekstrakcija donjih prvih premolara i primena donjeg i gornjeg fiksnog aparata za nivelaciju donjeg i gornjeg zubnog niza, normalizaciju incizalne stepenice i dubine preklopa. Celokupna terapija je trajala dve godine. **Zaključak.** Prikazana kombinovana kamuflažna terapija postigla je zadovoljavajući rezultat uprkos lošoj prognozi zbog hiperdivergentnog rasta.

Ključne reči:
malokluzija; ortodontski aparati, dizajn; kefalometrija; zubni luk; lečenje, ishod.

Introduction

The Veltri screw, within the palatal construction, appeared as a logical consequence of the evolution of appliances for palatal separation [1]. The beginnings of palatal constructions are related to the treatment of rapid palatal expansion and they date back to 1860, when Angle, having treated posterior crossbite of a fourteen-year-old boy, applied for the first time one kind of a primitive palatal construction with a screw. In the years to follow, the use of these appliances gave different results. Since 1960 they have been equally used in European countries and the USA. By the end of the same decade, the most commonly used palatal construction was Biederman's separator. It consisted of 4 bands, cemented on

Correspondence to: Tatjana Perović, University of Niš, Faculty of Medicine, Sestre Baković 16/22, 18 000 Niš, Serbia.
E-mail: tatjana.tanic@gmail.com

maxillary first premolars and molars and steel extensions which connected the central hygienic palatal screw and the bands [2]. In recent years, there has been the tendency of perfecting palatal constructions in terms of the range of their indication area: primarily distalization and transversal movement [1–6]. The improvement has also been achieved in the construction of the screw: the uneven surface of the screw was replaced with the smooth one which facilitated the hygiene and reduced the possibility of irritation; better mechanic resilience with the help of laser soldering of extensions onto the central body [1–5]. In addition, the stability achieved by a better construction, enabled removing of the bands on premolars, which simplified the placement of the appliance and improved the aesthetic impression since the appliance cannot be seen while smiling or speaking. All the mentioned above broaden the use of palatal constructions in the therapy of teeth movement, which is difficult or not possible to achieve at all with different kinds of appliances [1–6].

An appliance according to Veltri differs from the appliances with screws for rapid palatal exansion (Hass, Hyrax). In fact, its anchorage is not reciprocal as device appliance for rapid palatal expansion. Only one (at monolateral screws [7]) or two legs (at bilateral and rotary type [8]) which are mobile, move the teeth. Other legs and the rest of the construction are static and by anchoring teeth it represents very stable anchorage [1].

Case report

A patient was male, 14-years-old, with long, narrow and noticeable asymmetrical face, of convex profile, asthenic constitution (Figure 1) unsuccessfully orthodontically treated two years before. Intraoral finding was: I class occlusion by reconstruction; right: crossbite and lateral open bite of a severe degree, only molars and central incisors, which in "tête-à-tête" were in contact; left: the lack of contact with antagonists from the upper lateral incisor to the second premolar and somewhat milder degree of crossing than on the right side. There was a complete lack of space for the upper left canine and mildly crowded lower dental arch (Figure 2). Functional finding was: labored breathing through the nose with unremarkable rhinological findings, but the patient had a habit of breathing through the mouth due to frequent infections in early childhood. Mastication was difficult due to the lowered number of functional occlusal units. There was macroglossia and infantile swallowing.

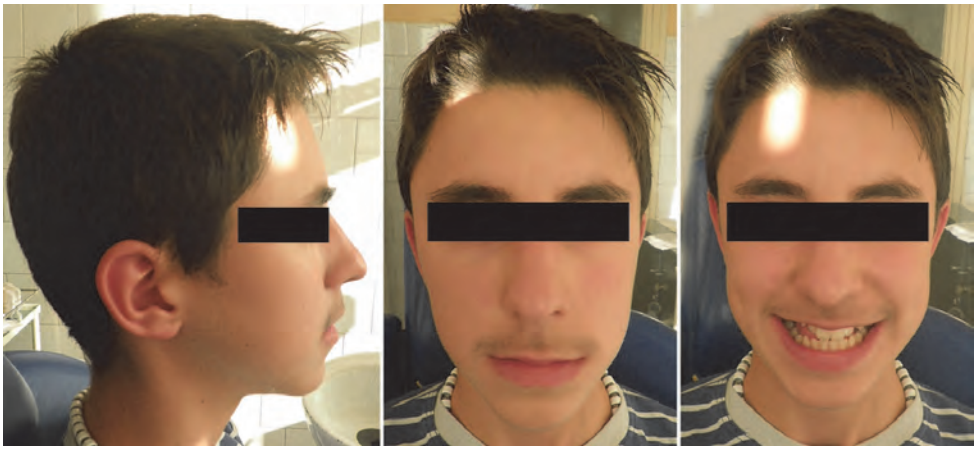

Fig. 1 – Pretreatment extraoral photographs.

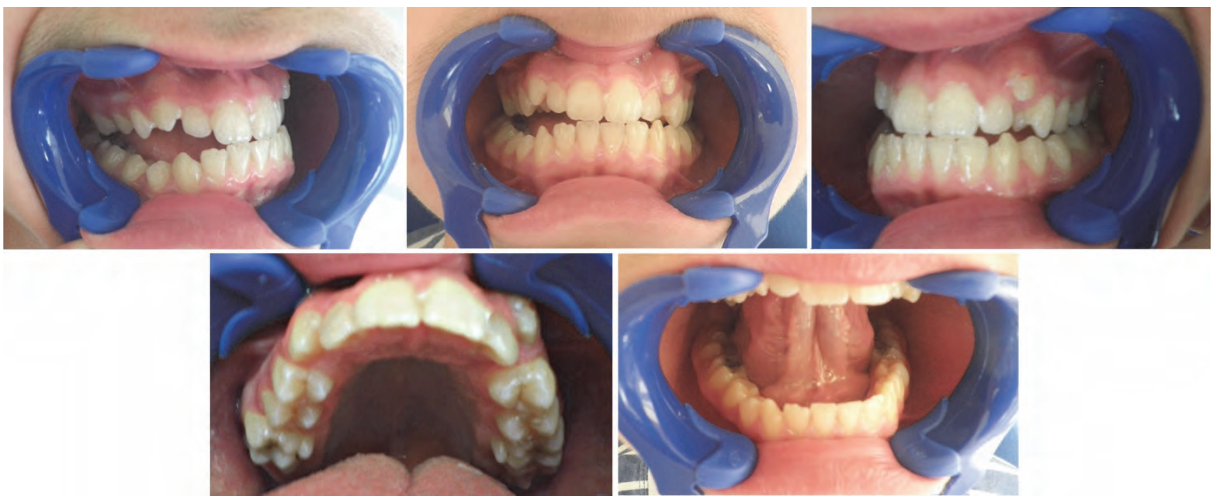

Fig. 2 – Pretreatment intraoral photographs.

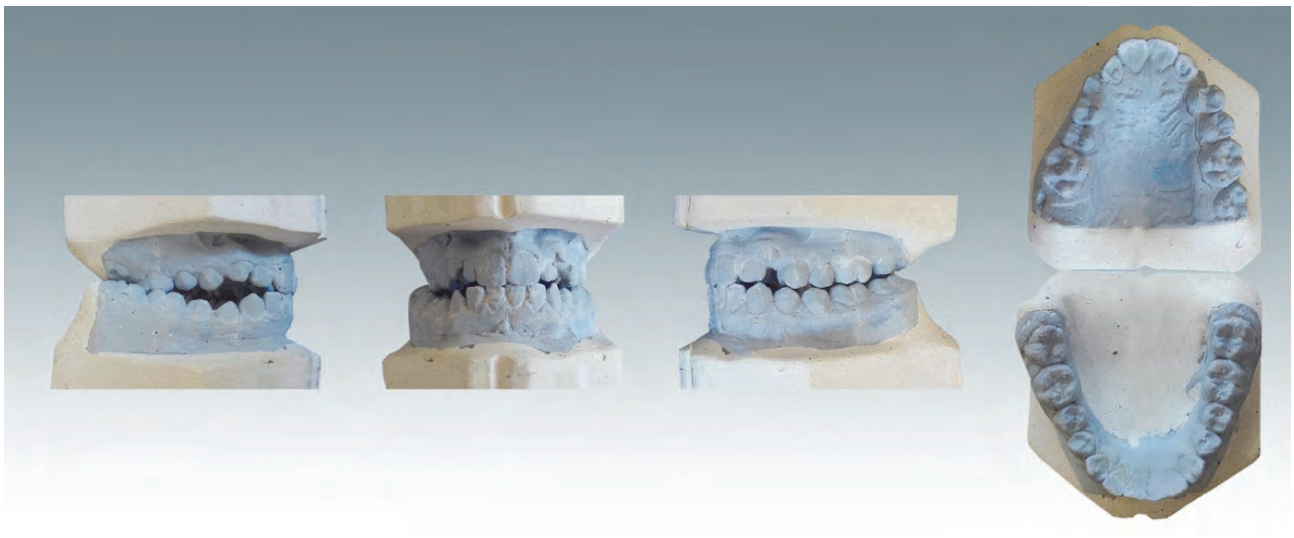

Fig. 3 – Pretreatment study models.

The analysis of the study model revealed (Figure 3): there was an asymmetrical narrowness of maxillary dental arch which caused discrepancy in the width of the upper and lower dental arch of 7 mm. Overjet and overbite values were zero (Table 1).

Cephalometric analysis (Figure 4) showed the increased basal (35^{o}) and gonial angle (144^{o}), followed by mesial gnathic and dentoalveolar relationship (Wits appraisal). There was a protrusion of lower incisors, with decreased interincisal angle (118^{o}). Linear measures showed contracted corpus of the upper jaw, in the direction of the base of the skull.

Analysis according to Steiner showed: bimaxillary retrognathism in class I skeletal relationship. The analysis of the growth indicated the growth in posterior rotation (Table 2). The overall findings suggested hypoplasia of the maxilla.

Table 1

The values of the frontal dentoalveolar parameters before and two years after the whole treatment

Features	Pre-treatment	Post-treatment
Incisor relationship	"Tête-à-tête"	Normal overjet
Overjet value, mm	0	2 mm
Overbite value, mm	0	3 mm
Midlines	Shifted	Co-incident

The orthopan analysis indicated the presence of crowdedness and unerupted third molars, which pointed to extraction therapy in the lower jaw (Figure 5).

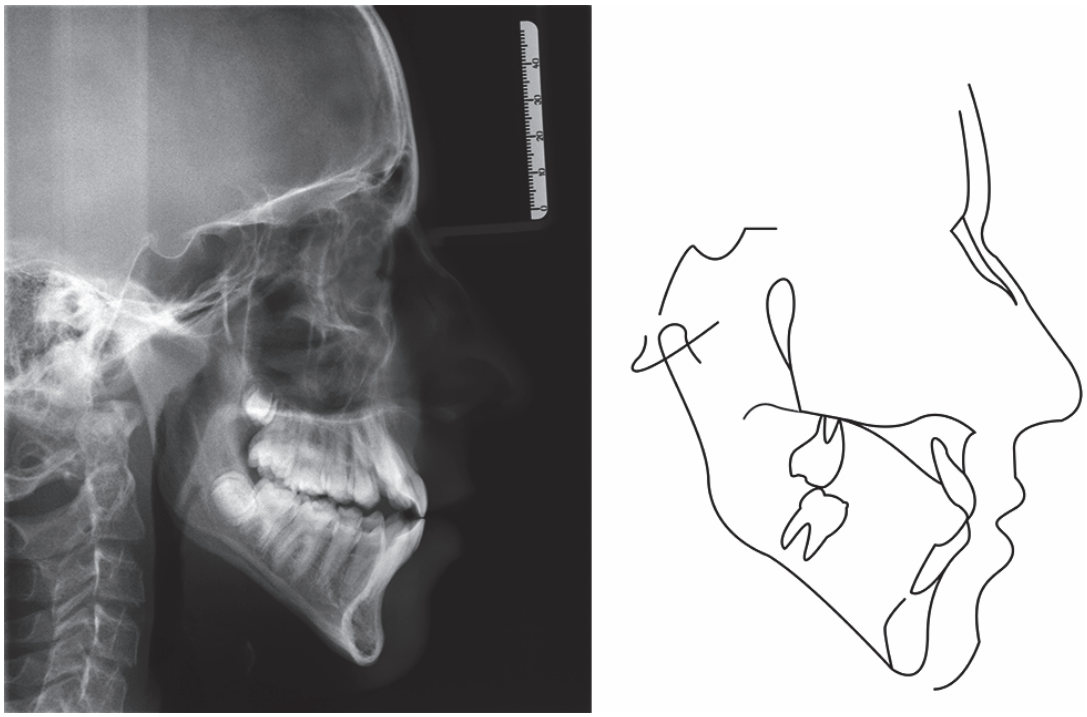

Fig. 4 – Pretreatment lateral cephalometric radiography and cephalometric tracings.

Table 2

The values of the cephalometric analysis before and two years after whole treatment

Angles	(Referent values)	Pre-treatment values	Post-treatment values
SNA, (˚)	82	80	80
SNB, (˚)	80	77	77
ANB, (˚)	2–4	3	3
SND, (˚)	76–77	75	76
I to NA, (˚)	22˚	22	36
I to NA, (˚)	4	4	8
i to NB, (˚)	25˚	34	25
i to NB, mm	4	10	6
Pg to NB, mm	4	2	3
I to i, (˚)	130˚–150˚	118	117
Occl/SN, (˚)	14˚	21	18
GoGn/SN, (˚)	32˚	46	46
SL, mm	51	46	44
SE, mm	24	21	17
FMA, (˚)	25˚	35	35
IMPA, (˚)	90˚	92	82
FMIA, (˚)	65˚	53	63
Wits app., mm	–1	2	–2
NMe, mm		117	118
SGo, mm		68	75
SGo/NMe, %	62–65	8.1	63.5
Y-axis, (˚)	59±4	61	62

SNA – Angle of sagittal maxillary position in relation to the cranial base anterior; SNB – Angle of sagittal mandibulary position in relation to the cranial base anterior; ANB – Angle of sagittal intermaxillar relationship; SND – Angle of sagital mandibulary position of the lower jaw (D – symphysis center); I to NA – Inclination of the upper incisors (in mm); I to NA – Inclination of the upper incisors (angle); i to NB – Inclination of the lower incisors (in mm); i to NB – Inclination of the lower incisors (angle); Pg to NB – Holdaway distance; I to i – Interincisal angle; Occl/SN – Inclination angle of the occlusal plane in relation to the cranial base anterior; GoGn/SN – Inclination angle of the madibular plane in relation to the cranial base anterior; SL – Sagittal position of the pogonion (in mm); SE – Sagittal position of the condyle (in mm); FMA – Francfort Mandibular Angle; IMPA – Incisor-Mandibular Angle; FMIA – Francfort Mandibular Incisor Angle; Wits app. – Wits assessment of sagittal intermaxilar relations; Nme – Anterior face height; Sgo – Postoeriof face height; Sgo/Nme – Posterior/anterior face height ratio; Y-axis –Angle between the Y-axis and Francfort plane.

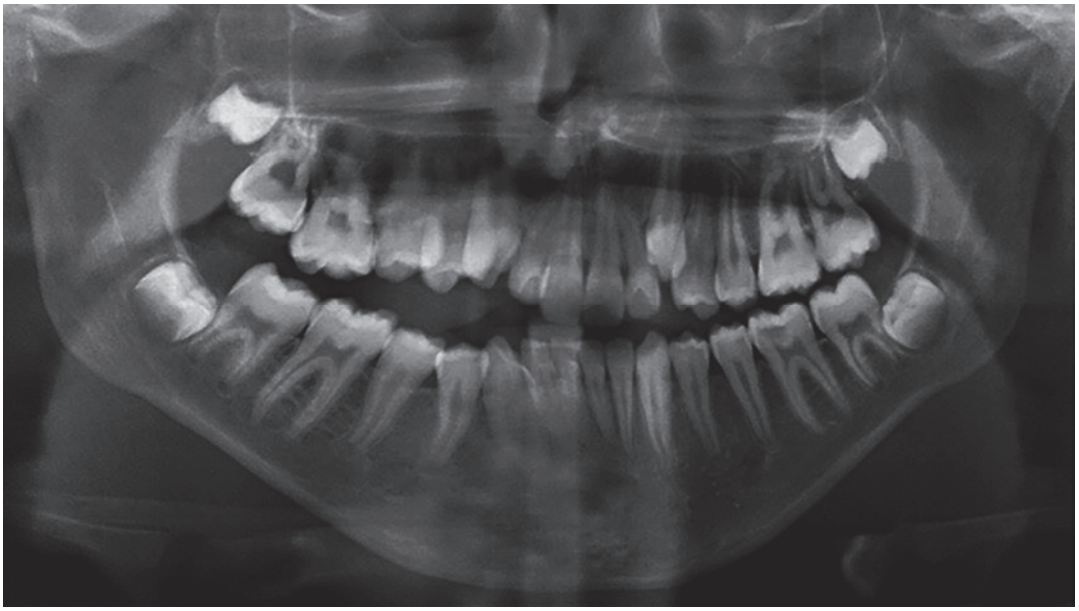

Fig. 5 – Panoramic radiograph before therapy.

The plan of the therapy included: 1) the adjustment of the widths of the upper with the lower dental arch by asymmetrical broadening by means of the Veltri appliance; 2) the extraction of the lower first premolars with the intention of dentoalveolar camouflage of the existing mesial gnathic and dentoalveolar skeletal relationship, relieving the crowdedness and retrusion of the lower front for the sake of establishing normal horizontal and vertical overlap of the incisors; 3) the alignment of dental arches, with the help of fixed appliances: to insert a left canine in the upper jaw in the dental line without extraction; in the lower jaw, close the postextraction space with retrusion of incisors and relieve crowdedness.

The priority in therapy was given to transversal adjustment of the upper and lower jaw by asymmetrical broadening. This effect was achieved by applying palatal construction with monolateral screw Veltri. The screw was activated every fifth day. By using this appliance, optimal width of maxilla was achieved with simultaneous correction of asymmetrical narrowness since only right lateral segment of the dental line was buccally moved. The buccal movement of the left lateral segment was slight, which was enough to correct a mild degree of crossing on the left side. The overall sum of expansion was 9 mm, out of which 2 mm of overcorrection for the sake of preventing therapeutic recidive (Figure 6). After the achieved effect, labial part of the rest was taken from the front teeth in order to place the upper fixed appliance (Figure 7). The Veltri appliance stayed in the mouth partly to stabilize the achieved result and partly to serve as a rest to the fixed appliance. At the same time, the lower first premolars were extracted and the lower fixed appliance was placed. Ten months after the placement of the fixed appliance, a satisfying therapeutic effect was achieved in the upper dental line. The upper fixed appliance was removed as well as the Veltri appliance. As a retention appliance, retention invisible appliance was made. In the lower jaw, the therapy lasted 6 months longer due to the closing of postextraction space and the correction of the middle of the lower dental line. After removing fixed appliance, retention invisible appliance was also applied. The acceptable occlusion was achieved intraorally in all three directions (Figure 8, Figure 9 and Table 1). There was a harmonization and improvement of facial contour profile (Figure 10). The cephalometric analysis showed harmonization of dentoskeletal relationships to the extent which the camouflage therapy allowed (Figure11, Figure 12 and Table 2).

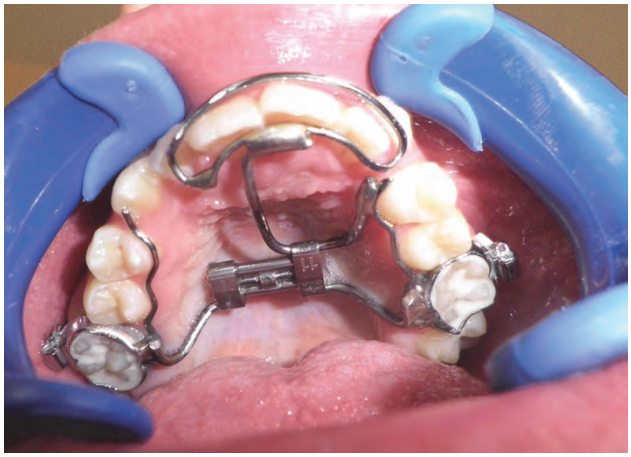

Fig. 6 – A progress occlusal view shows and adequate space created with the help of the Veltri appliance.

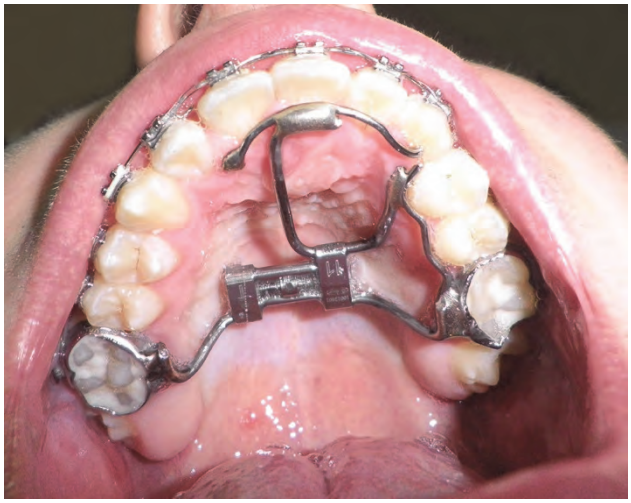

Fig. 7 – Occlusal view shows phase of combination therapy with the upper fixed appliance and reduced appliance according to Veltri.

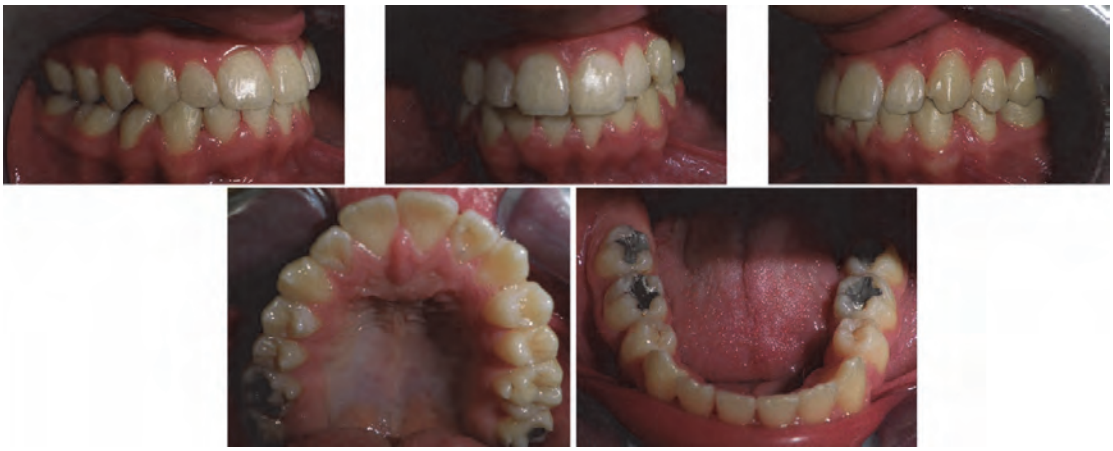

Fig. 8 – Post-treatment intraoral photographs, two years after the treatment.

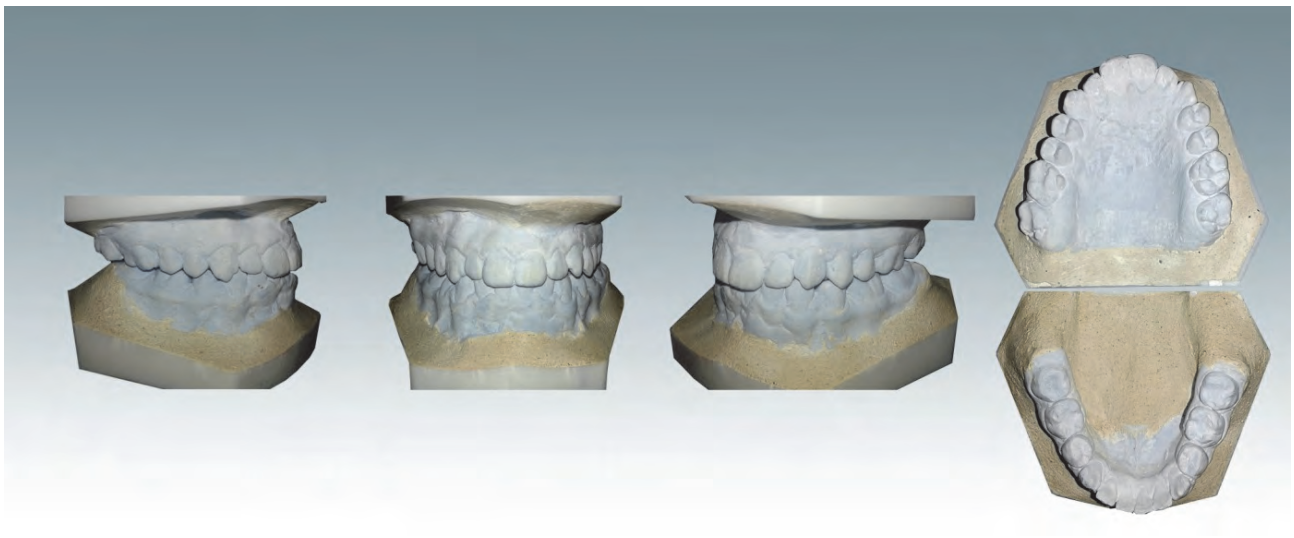

Fig. 9 – Post-treatment study models.

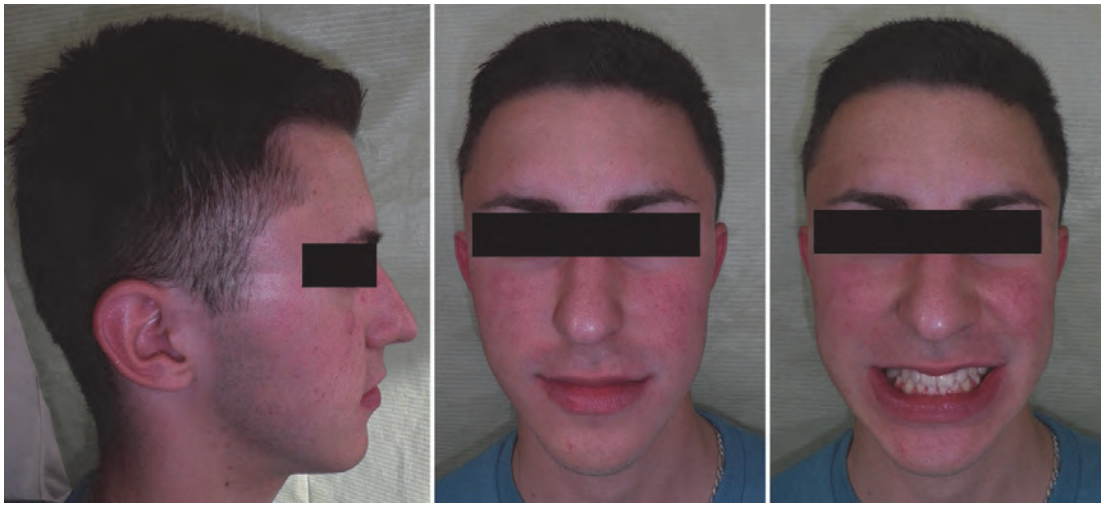

Fig. 10 – Post-treatment extraoral photographs.

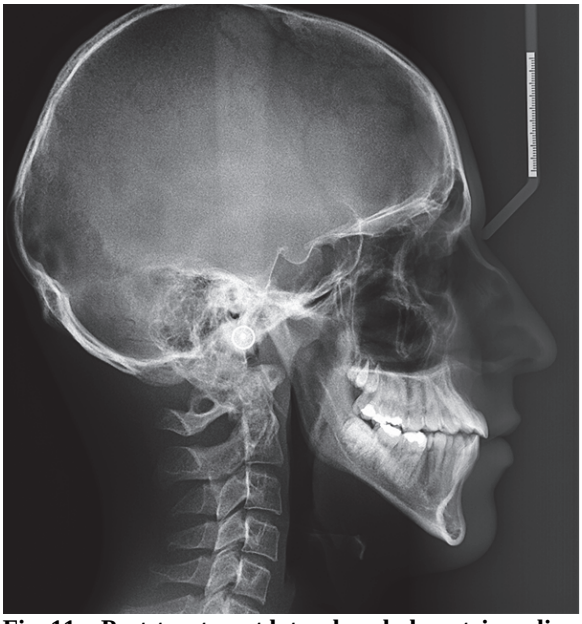

Fig. 11 – Post-treatment lateral cephalometric radiograph and cephalometric tracings.

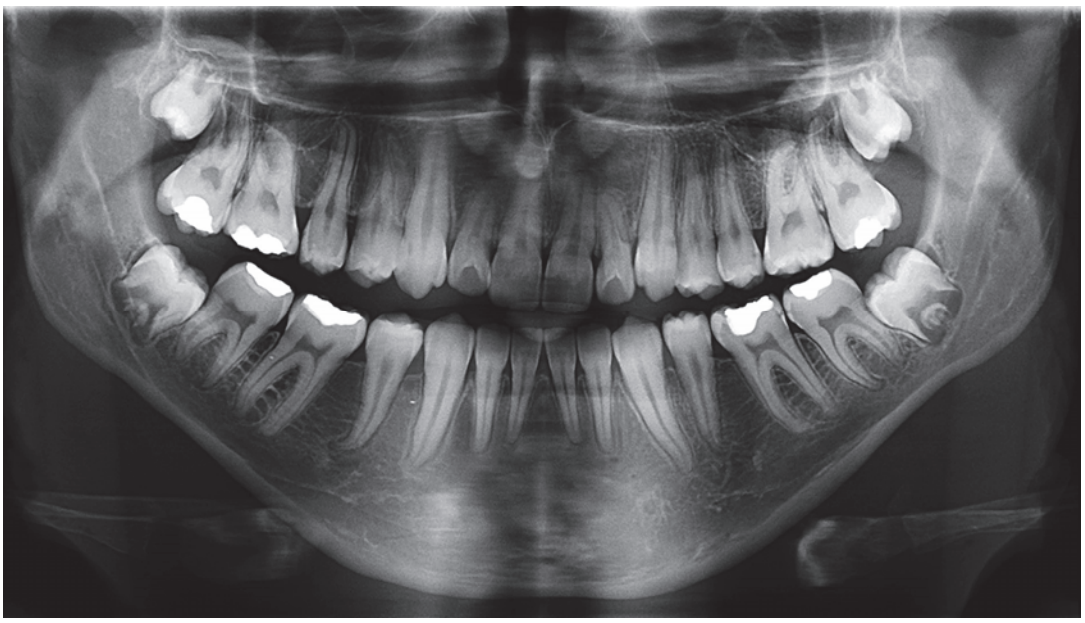

Fig. 12 – Panoramic radiograph after the therapy.

Discussion

Hypoplasia of maxilla, by itself, represents a severe orthodontic problem, which is usually noticeable as early as in childhood. It is considered to be of primarily hereditary etiology with the influence of oral respiration [9]. With age, in most cases, the deformity becomes more difficult, especially because there is a tendency towards infections of upper respiratory pathways [9]. Patients have mostly aesthetic motivation to solve this problem [10], but functional difficulties are not negligible either [9]. The upper incisors line is crowded, due to the underdevelopment of the maxilla in all three directions. What commonly accompanies this condition is impacted or retained canines [11], asymmetrical upper dental line [12], obligatory crossbite [12, 13], often followed by apertognathia, sometimes by mandibular deviation and mismatch of the middle of dental lines [13, 14], which further complicates the therapy of the already existing condition. The early therapy of hypoplasia of maxilla, has a great influence on the normalization of jaw relationship, especially if combination of facial mask Delair is applied [15] along with the rapid palatal expansion [9, 16]. If this period is skipped, with further progressing of irregularities and enlargement of sagittal skeletal discrepancy, it is hard to camouflage this problem, so it is most often treated orthodontically and surgically.

In the portrayed patient, the existing atypical and asymmetrical hypoplasia of the maxilla was further complicated by a difficult degree lateral apertognathia, which, along with hyperdivergent growth, represent a great therapeutic risk. The increased basal angle, by itself, implies extraction therapy. However, every extraction in the upper jaw would lead to additional consecutive collapse of the maxilla which would compromise the result of the therapy and produce deterioration of the existing sagittal dentoskeletal relationship. For that reason, compromised therapy was planned: the extension of the maxilla and extraction in the lower jaw. The protrusion of the lower incisors also justifies some extractions in the lower jaw.

The analysis of asymmetry by a transversal, indicated asymmetrical narrowness of the upper dental line so that symmetrical broadening was not considered. The amount of 7 mm of asymmetrical broadening would be impossible to achieve in practice with usual orthodontic procedures. For that reason, palatal construction with monolateral screw Veltri was applied. The construction is produced on the basis of monolateral screw Veltri. The fabric screw is adapted according to the relief and the depth of the palate on the cast model of the upper jaw. Then the bands on first molars that carry the entire construction are adapted. The adapted screw is then soldered onto the bands and the wire parts of the rest. The rest is secured with the tripod axle construction.

The result is achieved in one year at the longest. The duration of the therapy is influenced by the age of the patient and the amount of expansion needed to be achieved. The advantages of using this appliance are: the briefness of the therapy due to the continuity of treatment, aesthetic inconspicuousness, optimal therapeutic effect as well as the fact that after the accomplished expansion and during the use of a fixed appliance, the Veltri appliance remains to serve as a rest. The negative side is minimal discomfort for patients while eating, which is common in all fixed appliances.

Conclusion

The Veltri appliance with monolateral screw represents the appliance of choice in cases of the third degree unilateral crossbite in patients with permanent dentition. Its application enables relatively fast and successful correction of this irregularity with minimal discomfort for the patient.

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CASE REPORT

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Benign tumors of the heart: myxoma of the right atrium – A case report

Benigni tumori srca: miksom desne pretkomore

Saša Hinić*, Jelena Šarić*, Predrag Milojević[†‡], Jelena Gavrilović*, Tijana Durmić[‡§], Nebojša Ninković*, Branislav Milovanović[‡]*, Aleksandra Djoković[‡]*, Slobodan Mićović[†], Milosav Tomović[†], Marija Zdravković*[‡]

University Hospital Medical Center "Bežanijska Kosa", *Department of Cardiology, Belgrade, Serbia; [†]Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia; University of Belgrade, [‡]Faculty of Medicine, [§]Institute of Forensic Medicine "Milovan Milovanović", Belgrade, Serbia

Abstract

Introduction. Myxoma is the most common primary benign heart tumor. The most frequent location is the left atrium, the chamber of the heart that receives oxygen-rich blood from the lungs. Myxomas usually develop in women, typically between the ages of 40 and 60. Symptoms may occur at any time, but most often they are asymptomatic or oligosymptomatic for a long period of time. Symptoms usually go along with body position, and are related to compression of the heart cavities, embolization and the appearance of general symptoms. The diagnosis of benign tumors of the heart is based on anamnesis, clinical features and findings of the tumor masses by use of non-invasive and invasive imaging methods. Extensive surgical resection of the myxoma is curative with minimal mortality. Long term clinical and echocardiographic follow-up is mandatory. **Case report.** We reported a case of a 62-year-old male, presented with 15 days of intermittent shortness of breath, dizziness and feeling of heart palpitations and subsequently diagnosed with right atrial myxoma based on transthoracic echocardiography . The patient was emergently operated in our hospital. Long-term follow-up did not reveal recurrence. **Conclusion.** Our case was an atypical localisation of right atrial myxoma. Whether the intracardiac mass is benign or malignant, early surgery is obligatory in order to prevent complications.

Key words:
myxoma; heart neoplasms; diagnosis; echocardiography; echocardiography, transesophageal; histological techniques; cardiac surgical procedures; treatment outcome.

Apstrakt

Uvod. Miksomi su najčešći primarni benigni tumori srca. Najčešće su lokalizovani u levoj pretkomori koja prima krv bogatu kiseonikom iz pluća. U najvećem broju slučajeva sreću se kod osoba ženskog pola starosti od 40 do 60 godina. Simptomi se mogu pojaviti u svakom trenutku, ali su ovi tumori vrlo često asimptomatski ili oligosimptomatski tokom dužeg vremenskog perioda. Klinička slika zavisi od položaja tela i posledica je pritiska koji tumor vrši na srčane šupljine, embolizacije ili prisustva generalnih simptoma. Dijagnoza se postavlja na osnovu anamneze, kliničke slike i vizualizacijom tumora pomoću neinvazivnih i invazivnih dijagnostičkih procedura. Ekstenzivno hiruško uklanjanje je terapija izbora uz minimalnu stopu smrtnih ishoda. Dugotrajno kliničko praćenje je neophodno. **Prikaz bolesnika.** Prikazan je mušakrac star 62 godine, koji je 15 dana ranije imao povremeni osećaj kratkoće daha, vrtoglavice i lupanja srca. Dijagnoza miksoma desne pretkomore postavljena mu je na osnovu ehokardiografskog pregleda i tumor je hitno operativno uklonjen u našoj ustanovi. Tokom višegodišnjeg praćenja bolesnika nije uočen recidiv. **Zaključak.** Ovo je slučaj atipične lokalizacije miksoma u desnoj pretkomori. Bez obzira na to da li je intrakardijalna masa benignog ili malignog porekla, u cilju sprečavnja komplikacija neophodno je rano hiruško uklanjanje.

Ključne reči:
miksom; srce, naoplazme; dijagnoza; ehokardiografija; ehokardiografija, transezofagusna; histološke tehnike; hirurgija, kardijalna, procedure; lečenje, ishod.

Correspondence to: Marija Zdravković, University Hospital Medical Center "Bežanijska Kosa", Bežanijska kosa bb, 11 000 Belgrade, Serbia. E-mail: sekcija.kardioloska@gmail.com

Introduction

In general, though cardiac tumours can be malignant or benign, later group is more common. Literature data report that although most cardiac tumours are benign, they could have malignant potential due to secondary impaired cardiac function (congestive heart failure, inflow/outflow tract obstruction, conduction system involvement) or peripheral embolization [1].

Myxomas are the most frequent type of benign cardiac tumors in all age groups. Appart from them, other less common neoplastic tissues like rhabdomyomas or fibromas, are mostly seen in children. In all cases, a definitive diagnosis is important because of its unpredictive nature. Namely, some cardiac tumors can be malignant, or they can present metastasis from a primary tumor [2].

As the most frequent, myxomas arise usually from the endocardium. Due to its slow growth, in about 20% cases, they are asymptomatic or oligosymptomatic for long periods of time. In 90% of cases, it appears in the period of 30 to 60 years of age, prevailingly in the sixth decade of life, and 75% of sporadic myxomas occur in females [3].

In everyday clinical practice, the early detection is of a great importance. This fact is important because clinical presentation is non-specific and depends on localization, growth rate and size of tumor. The most common clinical presentations are the symptoms and signs of heart obstruction and embolization. Also, literature data indicates that benign cardiac tumors increase the risk of an ischaemic strokes [4, 5].

Macroscopically, myxomas are clearly limited, gelatinous consistence, reddish-blue or yellowish coloration. They grow, most frequently, in the atria, especially in the left atrium. Although the most cases are sporadic, approximately 10% are familiar and are transmitted in an autosomal dominant mode [4].

Case report

A 62-year-old man, admitted to the Coronary Care Intensive Unit, was presented with intermittent shortness of breath, dizziness, heart palpitations and progressive weight loss. The above symptoms were present for 15 days before admission. Personal history was positive for hypertension and smoking. Family history was positive for cardiovascular diseases. Upon clinical examination, the patient's heart rate was regular at 100 bpm, blood pressure was 150/100 mmHg, and body temperature was normal. The lips and finger nails were neither cyanotic nor clubbed. No thrills were detected in the precordial region. Lung sounds were normal, with soft systolic heart murmur (2/6) at Erb's point. Routine laboratory data showed high erythrocyte sedimentation rate [100 mm/h (reference range, < 20 mm/h)] and high D-dimer level (1,616 ng/mL). Electrocardiography (ECG) and chest X-ray were within normal limits (Figure 1). Transthoracic (Figure 2) and transesophageal echocardiography (Figure 3) revealed an occupying mass in his right atrium of approximately 5.3 x 4.6 cm and comfirmed moderate tricuspid obstruction.

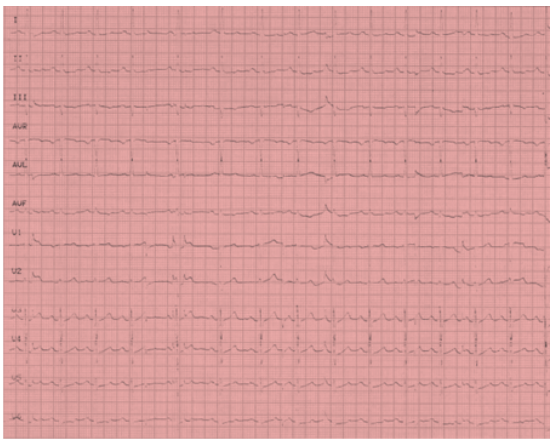

Fig. 1 – The patient's electrocardiogram (ECG) findings on admission.

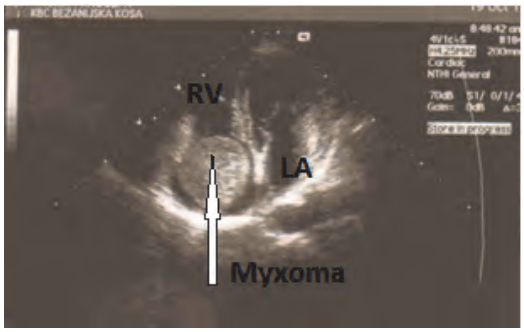

Fig. 2 – Echocardiogram shows the right atrial myxoma (highlighted). (LA – left atrium, RV – right ventricle).

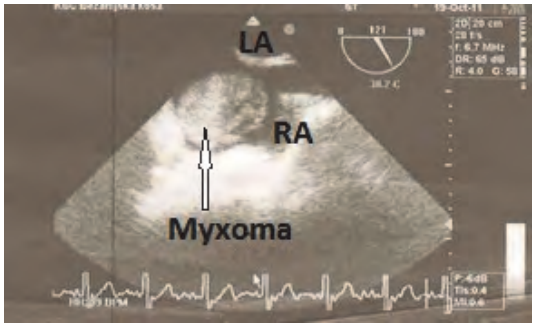

Fig. 3 – Transesophageal echocardiography revealed a large echo dense mass in the right atrium (LA = left atrium, RA = right atrium).

During his stay in the Coronary Intensive Care Unit he was hemodynamically stable with occasional episodes of paroxysmal supraventricular tachycardia.

He was scheduled for emergent surgical removal. Following preoperative preparation, the patient was taken for cardiac surgery. The heart was approached through median sternotomy. During surgery, the right atrium was opened and occupying mass was found in the posterior wall of right atrium and completely removed. Macroscopical examination of the mass revealed a smooth mass of 50 x 45 x 30 mm with a stalk arising from the posterior wall of the right atrium. The diagnosis was confirmed histologically.

Seven days after the operation, the patient was discharged from the hospital. Months and years later, further reexa-

minations, revealed no residual tumour. Three years after the operation, due to the permanently present atrial flutter type I, radiofrequency catheter ablation was done. The patient was returned to a sinus rhythm. On the last check-up, 5 years after the cardiac surgery, interatrial septum aneurysm was maintained (Figure 4).

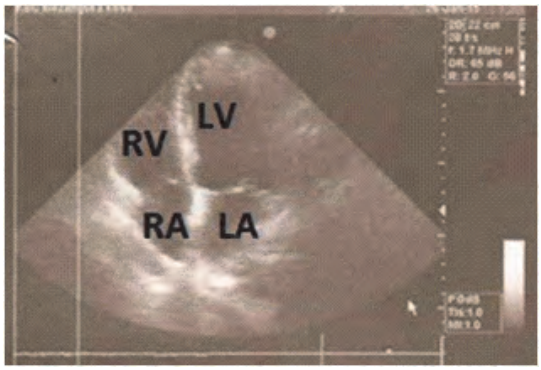

Fig. 4 – Four-chamber view of patient's transthoracic-echocardiographic study revealed interatrial septum aneurysm in a follow-up after 5 years. (LV – left ventricle; RV – right ventricle; RA – right atrium; LA – left atrium).

The study was approved by an Institutional Review Board and the patient gave his informed consent.

Discussion

Although most commonly cardiac myxomas arise from the interatrial septum, in our case the localization was rare – from the posterior wall of the right atrium [6].

Generally, myxoma is insidious primary cardiac tumor, commonly presented with nonspecific symptoms, such as fatigue, fever, myalgia, erythematous rash, arthralgia, weight loss, or laboratory abnormalities. These facts makes it difficult to diagnose and makes it potentially fatal if untreated [7].

Additionally, although they are regarded as mild proliferative lesions, with low metastatic potential and without modulation of tumoral suppressing genes or oncogenes, its malignancy may be determined by its specific behavior [8].

The literature data point on the fact that the presence of clinical symptoms depends on the tumor size. Namely, left atrial myxomas become symptomatic when they reach about 70 years, while right atrial myxomas may grow up twice this size before becoming symptomatic [9, 10].

Clinical presentation depends mostly on size, location and mobility of the tumor and thus may mimic arterial embolism or symptoms of cardiac, infectious, malignant and immune diseases, making its diagnosis more challenging [11].

Namely, the most common cause of complication is systemic embolization. It appears in 25%–50% of cases and can occur to any end organ leading to ischaemia and possible infarction [11]. The embolism end point depends on the location of myxoma and the presence of intracardial shunt.

Also, study conducted by Ha et al. [12] pointed on the association between polypoid cardiac myxomas and the occurrence of systemic embolism. Namely, the incidence of systemic embolization was significantly higher in patients with polypoid compared to round shaped myxomas. Because of previously mentioned fact, cardiac tumors should be in the differential diagnosis in every case of embolization.

Additionally, in some cases, benign tumors can manifest with arrhythmia, inflow/outflow obstructon, or even as a heart failure. Also, polypoid tumors more frequently prolapse through the mitral or tricuspid valve into the ventricle, causing the destruction of the annulus of valve leaflets [12].

Clinical significance also lies in the fact that they could be neovascularized by a branch of coronary artery, which can lead to bleeding in the myxoma with the progressive mass size increasement and consecutive blood flow obstruction [13].

Myxomas can produce numerous growth factors and cytokines, such as vascular endothelial growth factor that accelerates angiogenesis and results in tumor growth and increase the expression of proinflammatory cytokines [9, 14]. Thus, the period from the onset of the symptoms to the accurate diagnosis could be long and ranges from 1 to 126 months [15].

In some cases, cardiac myxomas can be associated with Carney complex, rare autosomal dominant syndrome, characterized with spooty skin pigmentation, endocrinopathy and endocrine or neuroendocrine tumors [16]. Although family members often have the same gene mutation, it is usually presented with distinct phenotypes as a result of various genetic and environmental factors. Recent studies identified mutations in the PRKAR1A gene that encodes the protein kinase A regulatory subunit 1-alpha (R1α) on chromosome 17q [16].

In terms of adequate diagnosis, two-dimensional echocardiography is in most cases sufficient. It provides an assessment of tumor location, size and mobility. On the other hand, transesophageal echocardiography is more sensitive, with better specificity and 100% sensitivity compared to transthoracic echocardiography. It also provides better resolution of heart chambers which makes it the imaging modality of choice [17]. Also, in patients older than 40 years of age it is very important to perform cardiac catheterization and angiography to exclude coexistent coronary artery disease or in order to evaluate neovascularization [9].

Immediately after the diagnosis is made, operative resection of the myxoma is the treatment of choice. After the surgical resection, diagnosis is comfirmed by histologic examination. Namely, myxomas are characterized by the presence of myxoid stroma fulfilled with the lipid cells [18]. Surgery is usually safe and curative, with an early postoperative mortality below 2% [19]. Complications are very rare and mostly presented as a postoperative atrial fibrillation, postsurgical neurologic complications or as it can be associated with unprofessional interatrial septum revision and consecutive bleeding which require exploration [19, 20].

Generally, the possibility of myxoma reccurence is rare, except in younger patients or in cases of Carney complex, where the possibility of the tumor recurrence is high. In these cases it is reasonable to do a biannual transthoracic echocardiography [12]. According to recent literature data, in all other cases, because of rare myxoma recurrence rate, continuous annual echocardiography is called into question [21].

Conclusion

We presented a case of a patient with an atypically located myxoma arising from the posterior wall of the right atrium.

Diagnosed by echocardiogram and after emergently surgical removal comfirmed by histological analysis.

Although rare, cardiac myxomas are the most common benign cardiac tumors. Its appearance is very important since it has nonspecific symptoms. Given the high embolic potential of myxomas, surgical removal is always the best treatment option. Prior the surgery, transthoracic echocardiography represents the single and the most important diagnostic method which gives the accurate anatomic information and identifies a precise surgical strategy.

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CASE REPORT

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Difficulties in diagnosis of tuberculosis without bacteriological confirmation in a 15-year-old boy after the contact with a patient with tuberculosis – A case report

Teškoće u dijagnostici tuberkuloze bez bakteriološke potvrde kod 15-godišnjeg bolesnika dobijene kontaktom sa obolelim od tuberkuloze

Gordana Kostić*†, Raša Medović*†, Slavica Marković*†, Zorica Rašković*†, Zoran Igrutinović*†, Vojislav Ćupurdija†‡, Marina Petrović†‡

Clinical Center Kragujevac, *Clinic for Pediatrics, ‡Center for Pulmonary Diseases, Kragujevac, Serbia; University of Kragujevac, †Faculty of Medical Sciences, Kragujevac, Serbia

Abstract

Introduction. After the contact with a patient suffering from tuberculosis (TB), previously healthy children have 1%–16% possibility to develop the disease. TB diagnosis in children is not easy to confirm so 15%–25% of cases remain undiagnosed. **Case report.** A 15-year-old-boy was hospitalized with productive cough, pain in the right flank area, fever, and fatigue, loss of appetite and night sweats. One of the boy's uncles was cured of tuberculosis, another uncle had active tuberculosis and both of them were in contact with the boy, but they did not live in the same household. During the physical examination, the child was febrile, with dyspnea, pale, with profuse sweating, debilitate. BCG (Bacillus Calmette – Guérin) scar was present. The auscultatory findings of the lungs showed quiet breathing from the scapula to the right lung base and chest radiography suggested massive right sided pleuropneumonia. The parameters of the inflammation were high and *Mycobacterium tuberculosis* (MTB) was not found in the samples of sputum and gastric lavage. Pleural puncture revealed exudative nature in the aspirated fragment. Cytology was nonspecific, the MTB was not found and the planted surfaces on Lowenstein-Jensen remained sterile. Tuberculin skin test (TST) – Mantoux was positive (+ 10 mm), Interferon Gamma Release Assay (QuantiFERON-TB GOLD In-Tube) was negative. The boy was unsuccessfully treated with broad spectrum antibiotics. By video-assisted thoracoscopy, the pleural tissue clip confirmed the benign chronic granulomatous process, while histochemical staining did not show MTB. The treatment with anti-TB medication led to clinical and radiographic recovery. The boy is now in good general condition, without consequences of the disease. **Conclusion.** This case report pointed out the importance of risk factors and difficulties in diagnosing TB in children.

Key words:
tuberculosis, pulmonary; diagnostic techniques and procedures; diagnosis, differential; bacteriology; drug therapy.

Apstrakt

Uvod. Nakon kontakta prethodno zdrave dece sa obolelim od tuberkuloze (TB) mogućnost razvoja bolesti iznosi 1%–16%. Dijagnozu TB kod dece nije lako potvrditi tako da 15%–25% slučajeva ostaje nedijagnostikovano. **Prikaz slučaja.** Dečak star 15 godina hospitalizovan je zbog produktivnog kašlja, bola u desnom slabinskom predelu, povišene temperature, malaksalosti, gubitka apetita i noćnog znojenja. Jedan dečakov ujak izlečen je od tuberkuloze, drugi ujak ima aktivnu tuberkulozu, obojica su bila u kontaktu sa dečakom, ali ne žive u istom domaćinstvu. Pri fizikalnom pregledu bolesnik je bio febrilan, dispnoičan, bled, sa profuznim znojenjem, adinamičan. BCG (Bacillus Calmette – Guérin) ožiljak je prisutan. Radiografija grudnog koša ukazivala je na masivnu desnostranu pleuropneumoniju. Parametri inflamacije su bili povišeni, a u uzorcima sputuma i gastričnog lavata nije nađen *Mycobacterium tuberculosis* (MTB). Pleuralnom punk-

Correspondence to: Gordana Kostić, Clinical Center Kragujevac, Pediatric Clinic, Zmaj Jovina 30, 34 000 Kragujevac, Serbia.
E-mail: goca kostic58@gmail.com

cijom, utvrđeno je da je punktat eksudativne prirode. Citološki pregled bio je nespecifičan, nisu nađeni MTB i sve zasejane podloge na kulturi Lowenstein-Jensen ostale su sterilne. Tuberkulinski kožni test Mantoux bio je pozitivan (+10 mm). Interferon Gamma Release Assay (QuantiFERON-TB GOLD in-Tube) bio je negativan. Dečak je bez uspeha lečen antibioticima širokog spektra dejstva. Video–asistiranom torakoskopijom, isečak tkiva pleure potvrdio je da se radi o benignom, hroničnom granulomatoznom procesu, dok histohemijskim bojenjem nisu viđeni MTB. Započeto lečenje antituberkuloticima dovelo je do kliničkog oporavka i radiografske regresije. Dečak je sada dobrog opšteg stanja, bez sekvela bolesti. **Zaključak.** Ovaj prikaz slučaja ukazao je na značaj faktora rizika, kao i otežano dijagnostikovanje TB kod dece. Auskultacijom pluća ustanovljeno je nečujno disanje od skapule desno ka bazi pluća.

Ključne reči:
tuberkuloza pluća; deca; dijagnostičke tehnike i procedure; dijagnoza, diferencijalna; bakteriologija; lečenje lekovima.

Introduction

Infection with *Mycobacterium tuberculosis* (MTB), does not always lead to active illness [1–5]. After the contact of previously healthy children with people suffering from tuberculosis (TB), the possibility of development of active illness is 1%–16%. The highest risk is during the first two years after the infection and declines over time [6–10]. The symptoms of TB may arise from the respiratory tract, the cough longer than 2–3 weeks, chest pain, shortness of breath, blood coughing, or non-specific loss of appetite, fatigue, prolonged fever, chills, weight loss, night sweats [1–5, 11]. In the pediatric intensive care units, 19% of all severe pneumonia develop from TB [12, 13], and the incidence of TB pleural effusion is about 16% of all pleural effusions [14, 15].

The diagnosis is based on history, physical examination, radiographic findings (chest X-ray and computed tomography), microscopy and culturing bacteria from sputum, gastric lavage, bronchoscopy, pleural puncture, or video-assisted thoracoscopy (VATS) of obtained content or tissue view, tuberculin skin test (TST), Interferon Gamma Release Assay (IGRA), response to the use of broad-spectrum antibiotics and physician assessment [1–5, 12–18]. Because of the difficulty in obtaining adequate samples for laboratory tests, the diagnosis is not easy to confirm; 15%–25% of all suspected cases remain undiagnosed despite the use of combined diagnostic methods [1, 5, 16].

Case report

A 15-year-old boy was hospitalized at the Clinic of Pediatrics, Clinical Centre Kragujevac due to five-day productive cough, the pain in the right flank area and elevated temperatures up to 39°C. Twenty days before the admission to hospital the boy had a sense of fatigue, malaise, loss of appetite and night sweats.

History taking revealed two uncles with tuberculosis. One uncle was cured successfully three years ago, and the other was currently under treatment. The boy lived with his parents of middle socioeconomic status, away from the uncles, but he was in frequent contacts with them.

On the day of admission the boy's body mass was 49 kg (-5 kg), height 164 cm (P_{25}) with body mass index 18.22 kg/m^2 (P_{25-10}). He was aware, debilitate, febrile (38.2°C), pale, with dyspnea and profuse sweating. Post vaccine Bacillus Calmette-Guerin (BCG) scar of 3 mm was present.

Chest respiratory movements were regular with a slight lag from the right. The auscultatory finding showed from the middle of the scapula to the right lung base treacherous muffled sound, with a weakened pectoral fremitus and inaudible breathing; oxygen saturation (SaO_2): was 93%.

Peripheral blood laboratory analyses showed: erythrocyte sedimentation rate 60 mm/h; hemoglobin – 107 g/L; leukocytes – 17.8 × 10^9/L (lymphocyte dominance 10.2 × 10^9/L); C-reactive protein (CRP) – 102.5 mg/L; fibrinogen – 5.151 g/L. Elevated levels of total cholesterol (5.91 mmol/L), low density lipoprotein (LDL) cholesterol (4.01 mmol/L) and triglycerides (2.32 mmol/L) were found. Other biochemical analyses were within normal range. IgM serologic result of the *Mycoplasma pneumoniae* was negative. Humoral immunity (IgM, IgG, IgA) showed normal values.

Chest X-ray showed large right sided pleural effusion up to the height of the 4th rib front corners (Figure 1).

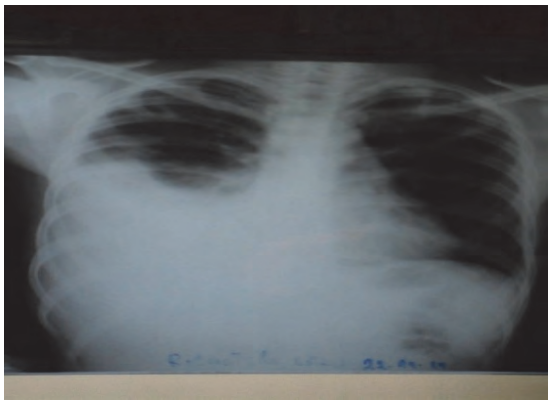

Fig. 1 – Chest radiography (X-ray) – large pleural effusion to the height of the front corners of IV ribs.

Ultrasonography of the abdomen showed that liver, gall bladder, pancreas, spleen, kidneys and bladder were normal. No free fluid or enlarged lymph nodes in the abdomen were found. Ultrasound examination of the heart showed the pericardium without pleural effusion. Due to radiographically revealed pleural effusion, the pleural puncture was performed and 500 mL of serohemorrhagic content was evacuated. The material was sent to biochemical, cytological and bacte-

riological analyses, staining by Zeihl-Neelsen method and seeding in Lowenstein–Jensen medium.

Cytological examination showed gray content with non-structured smear but with erythrocytes, lymphocytes, rare polymorphonuclear leukocytes and only a few macrophages and mesothelium cells.

Biochemical examination confirmed the exudative content in the pleural cavity. The ratio of proteins in the pleural aspirate/serum > 0.5 (59/80 g/L = 0.74), the ratio of lactate dehydrogenase (LDH) in the pleural aspirate/serum > 0.6 (897/362 U/L = 2.48) were found.

Neelsen staining did not show *Mycobacteria*, and planted surfaces on Lowenstein-Jensen medium remained sterile.

Tuberculin skin test (Mantoux) was positive (+10 mm), IGRA (QuantiFERON-TB GOLD in-Tube) test was negative. The treatment began with administration of broad spectrum antibiotics (cephalosporins of the third generation, macrolides, carbapenems), and bronchodilators, as well as the use of symptomatic treatments. After two weeks of treatment, there was a partial improvement of the clinical picture: reduction in symptoms (temperature decreased to 37.5°C, with less dyspnea), together with partial regression of radiological pleural effusion on the right side. Due to incomplete radiographic regression of pleural effusion and maintenance of subjective symptoms (night sweats, loss of appetite) VATS was indicated.

With this procedure, the clip of pleural tissue was taken for histological and Ziehl-Neelsen analyses. According to the histological analysis, benign, chronic granulomatous process was found, with parts of caseous foci, which could morphologically be tuberculosis process (according to the findings of pathologist at Military Medical Academy, Belgrade, Serbia).

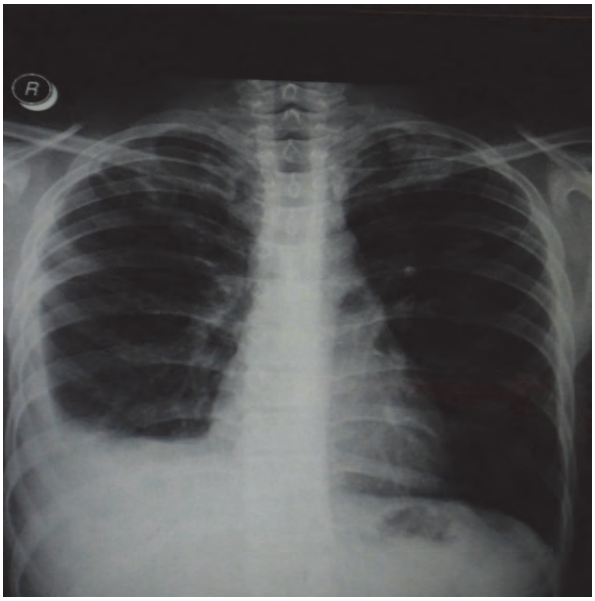

Fig. 2 – Chest radiography (X-ray) two weeks after the introduction of antituberculosis treatment.

Taking into consideration the above findings, and especially the fact that the boy had been in contact with TB patients, clinicians decided to begin anti-tuberculosis treatment following the standard regime. The therapy included the first line drugs (isoniazid, rifampicin, pyrazinamide, ethambutol) and orally administered corticosteroids, H_2-receptor blockers and vitamin B_6. After a week of antituberculosis treatment, significant clinical, laboratory and radiographic improvement was found (Figure 2), with the loss of symptoms (no fever, no night sweats), improvement of physical findings in the lungs and reduction of biohumoral inflammation parameters (SE – 10 mm/h, CRP – 1.9 mg/L).

After two months of initial antituberculosis treatment the chest radiography showed significant radiological regression of pleural effusion (right costophrenic sinus shaded with small shadow estuary, horizontal posterior was shown in the lateral part, the suspected small traction of the apical pleural on the right) (Figure 3).

After continual phase of the treatment (during four months and administration of two drugs), chest radiography showed complete radiographic regression of right sided pleural effusion.

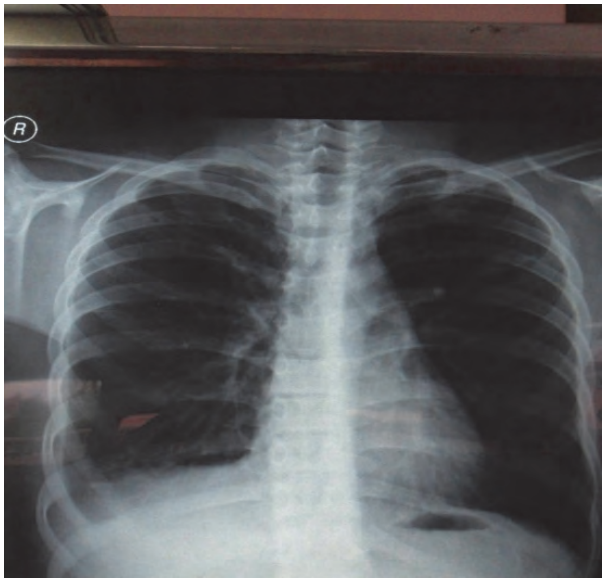

Fig. 3 – Chest radiography (X-ray) after two months of antituberculosis treatment.

Discussion

TB in children makes 5%–20% of the total, and about 74,000 children in the world die every year [4, 19, 20]. According to Serbian Report on Tuberculosis, the morbidity rate in all age groups is 17 : 100,000, whereas the morbidity rate in children population is 6.425 : 100,000 with specific morbidity rate of 8.5 : 100,000 in the population aged 15–19 years [21]. After the contact with an adult suffering from pulmonary TB, the possibility of developing active disease is created in 1%–16% of previously healthy children with preserved immunity [6–10]. Studies have shown that the different countries have different percentage of illness after the contact. The highest percentage of TB after the contact was found in the Netherlands study [6] whereas much lower inciden-

ce of 3% was reported in a Chinese study [7], followed by considerably lower results in a Vietnam study (0.7%) [8] and 0.3% in a Norway study [10]. These data indicate that the contact with the patient is not sufficient per se for the development of disease. The risk increases due to prolonged exposure, higher concentration of infected droplet nuclei MTB in the air, less volume of the joint space, lack of ventilation and direct sunlight and social factors [9, 22, 23].

Most of the predisposing factors were present in our patient and recent contact with the TB patient was among the most important ones. The risk of development of the disease is highest during the first year of the contact and ranges up to 50% [6–10, 22, 23]. In the incidence of TB, genetic predisposition has an important role as well [24, 25].

Although the diagnosis of TB in children is more difficult, according to Marais et al. [11] classic clinical symptoms may indicate TB in children in almost 63% cases.

When parameters of increased inflammation are added to positive X-ray, the percentage of suspected TB increases to 80% [1, 4, 5, 16].

In 87% cases, non-invasive methods such as chest radiography with elevated inflammatory parameters and a positive Tuberculin skin test confirm the diagnosis of tuberculosis [26]. Thorax computered tomography (CT) is also recommended in cases of suspected TB. The boy's mother refused this proposed diagnostic procedure due to the risk of radiation, and several studies described the possibility of negative TB diagnostics after CT [1, 2, 4, 5, 16], there fore we did not perform it. After the contact with a TB patient, Tuberculin Skin Test is positive in 50%–60% patients and the almost same percentage is with IGRA test. If the two tests are combined, the percentage is slightly higher [1, 2, 16, 17, 26, 27]. After special staining, the percentage of positive findings of sputum smear microscopy in children ranges from 1%–15%, and Lowenstein sown crops are positive in 10%–20% (and in best cases in children, it is 50%) [1, 2, 5, 16, 26]. Some researchers found that better results were obtained from seeding gastric lavage in comparison to sputum [28]. The test of nucleic acid amplification as well as the Xpert MTB/RIF (susceptibility testing for rifampicin) test are not performed in our center due to technical reasons, although a large group of experts in the countries with high prevalence of TB, such as India, no longer recommend them in diagnostics of TB in children [1, 19].

It is recommended that TB is examined in each bronchopneumonia with pleural effusion. In addition, in TB pleurisy the inflammation is located in lungs in 20%–40%, which is not initially recognized in the lung radiography. For larger pleural effusion, the thoracentesis is indicated due to diagnostic and therapeutic importance. In pleural aspirate during the cytological examination the mononuclear cells are found in 90%, and in 92% the definite diagnosis of this disease is histologically confirmed. In children, TB pleurisy has a good prognosis and it usually resolved without consequences [12, 14, 15]. This was the case with the patient we presented.

During the hospitalization of a child with severe pleuropneumonia and period for collecting the test results for confirmation of TB, it is necessary to administrate the initial antibiotic therapy in addition to other measures [12, 13]. Poor therapeutic response to broad spectrum antibiotics is the new protocol considered as another diagnostic guide for TB [1–5, 19, 20].

After all diagnostic methods were exhausted in our patient, and when the treatment with broad-spectrum antibiotics began, the X-ray shadow of pleural effusion was still present; therefore it was decided to do VATS in order to confirm histopathological process. VATS was introduced as a diagnostic method for TB 15–20 years ago, but in the developing countries its application began just ten years ago [29, 30]. Even larger centers than ours do not apply it as a diagnostic method for TB, especially with children population [18, 30]. The indication should be considered in each particular case.

Despite the absence of TB bacteriological confirmation, due to the clear persistence of risk factors for developing TB, clinical symptoms, maintenance of elevated markers of inflammation besides antibiotic treatment and lung radiographic findings, antituberculosis therapy was administered and led to healing of the child.

There is no gold standard for TB diagnosis in some cases, and, sometimes positive clinical findings and epidemiological data are enough for beginning of antituberculosis treatment. Despite the use of the old and introduction of the many new diagnostic procedures, primarily the development of immunological tests (IGRAs false negative results in children according to some new data from literature), the global burden of TB diagnosis still remains significant [2, 4, 31, 32, 33]. In the presented case only the clinical suspicion of TB, a border level of TST (the criteria for positivity is 6–10 mm for BCG vaccinated patients) and chest radiography verifying severe bronchopneumonia were the criteria for introduction of antituberculosis therapy [1–5, 19, 20].

Conclusion

This case report pointed out the importance of risk factors and difficulties in diagnosing TB in children.

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CASE REPORT

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Epithelioid sarcoma of femoral nerve – A case report

Epiteloidni sarkom femoralnog nerva

Dejan Ivanov*†, Mirjana Živojinov†‡, Milan Ranisavljević†§

Clinical Center of Vojvodina, *Clinic for Abdominal, Endocrine Surgery and Transplantation, †Department for Pathology, Novi Sad, Serbia; ‡Oncology Institute of Vojvodina, Sremska Kamenica, Serbia; University of Novi Sad, §Faculty of Medicine, Novi Sad, Serbia

Abstract

Introduction. Epithelioid sarcoma is a slow-growing malignant soft tissue tumor and occurs approximately in 1% of all soft tissue malignant tumors. This case report describes epithelioid sarcoma in femoral nerve and as we know it is the first described case of epithelioid sarcoma at this anatomical localisation. **Case report.** A 44-years-old female patient presented with strong pain in her left leg. On magnetic resonance imaging (MRI), tumor was presented as a node 8 cm in the diameter at left femoral nerve between ileopsoas and iliacus muscle, without infiltration of muscle fascia and tendons. Four enlarged lymph nodes described in left iliac fosa, were suspected on secondary tumor deposits. We preformed radical surgical excision of the tumor and femoral nerve transection with local lymp hnode disection. Histopathological analysis revealed epithelioid sarcoma, and tumor stained highly positive for anti-pan cytokeratin antibody (AE1/AE3), vimentin, cancer antigen 125 (CA125), anti-cytokeratin antibody (MNF116), hematopoietic progenitor cell antigen (CD34) and epithelial membrane antigen (EMA) markers. After the operation patient recived radiotherapy without chemotherapy. Six months postoperatively, there was no evidence of local relapses or distant metastases. **Conclusion.** Initial wide surgical resection and adjuvant radiotherapy is beneficial in treatment of epitheloid sarcoma.

Key words:
sarcoma; soft tissue neoplasms; femoral nerve; diagnostic techniques and procedures; surgical procedures, operative; radiotherapy.

Apstrakt

Uvod. Epiteloidni sarkom je redak, sporo rastući maligni tumor mekog tkiva i pronalazi se u oko 1% svih mezenhimnih tumora. Prikazujemo slučaj epiteloidnog sarkoma femoralnog nerva, koji je prema našim saznanjima, prvi ovakav opisan slučaj. **Prikaz bolesnika.** Bolesnica stara 44 godine, primljena je na pregled sa jakim bolovima u levoj nozi. Na snimku magnetne rezonance tumor se prezentovao kao nodus dimenzija 8 cm na levom femoralnom nervu, između bočnog slabinskog i ilijačnog mišića, bez infiltracije mišićnih fascija i tetiva. Četiri uvećana limfna čvora, opisana u ilijačnoj jami, bila su suspektna na prisustvo sekundarnih depozita. Urađena je radikalna hirurška ekscizija tumora i transekcija nerva sa lokalnom limfadenektomijom. Patohistološka analiza potvrdila je dijagnozu epiteloidnog sarkoma, a tumor je pokazivao izrazitu imunohistohemijsku pozitivnost na anti-pan citokeratin antitelo (AE1/AE3), vimentin, karcinomski antigen 125 (CA125), anti-citokeratin antitelo (MNF116), antigen progenitorskih ćelija hematopoeze (CD34) i epitelni membranski antigen (EMA). Nakon operacije bolesnica je primila radioterapiju bez hemioterapije. Šest meseci nakon operativnog lečenja kod bolesnice nije bilo lokalnog recidiva i udaljenih metastaza. **Zaključak.** Inicijalna široka resekcija i adjuvantna radioterapija je ključna u tretmanu epiteloidnog sarkoma.

Ključne reči:
sarkomi; meka tkiva; n. femoralis; dijagnostičke tehnike i procedure; hirurgija, operativne procedure; radioterapija.

Introduction

Epithelioid sarcoma (ES) is an uncommon malignant soft tissue tumor. It is a slow-growing tumor and occurs approximately in 1% of all soft tissue malignant tumors. It is mostly found in young adult male patients.Proximal and distal type of ES was described. Proximal type is located in subcutaneous tissues, muscular fascia or tendon sheaths of the upper extremities. Distal type has the same anatomical localisation but on distal limbs. Rare cases have been reported in the pelvis, vulva, penis, and spine [1–5].

Correspondence to: Milan Ranisavljević, Clinical Center of Vojvodina, Clinic for Abdominal, Endocrine Surgery and Transplantation, Somborska 8a, 21000 Novi Sad, Serbia. E-mail: ranke82@gmail.com

This case report describes ES in femoral nerve and it is the first case of ES at this anatomical location on the basis of avaible literature data.

Case report

A 44-years-old female patient was first examinated by physiatrist, because of strong pain in her left leg. After unsuccessful physiatrist treatments, her doctor decided to perform magnetic resonance imaging (MRI) of small pelvis and lumbal spine.

On MRI tumor was presented like a node of 8 cm in diameter at the left femoral nerve between ileopsoas and iliacus muscle, without infiltration of muscle fascia and tendons. Four enlarged lymph nodes were described in left iliac fosa, with a suspicion of secondary tumor deposits. After additional diagnostic procedures (ultrasonography – US, blood analysis), she was referred to general surgeon with presumed diagnosis of left femoral nerve tumor – retroperitoneal tumor. In her medical history there were no chronic diseases. She had no personal or family history of cancer.

After standard preoperative preparation, the patient underwent operative treatment. Intraoperatively, we found a small amount of cloudy ascites intraperitoneally. After section of peritoneum in left iliac fosa we found the tumor mass. Tumor was 8 x 4 cm in diameter and it was located inside femoral nerve with partial infiltration of ileopsoas muscle fascia. Four enlarged lymph nodes clinically suspected on metastasis were found near the tumor.

After surgical preparation of the left femoral nerve and tumor deliberation from nearby structures we performed dissection of the nearby lymph nodes which we sent to histopatological fast frosen anaylsis (HP *ex tempore*). *Ex tempore* analysis revealed malignant mesenhimal tumor.

After consultation with neurosurgeon we decided to make nerve transection, proximal and distal from the tumor mass and partial resection of the left ileopsoas muscle with fascia. Surgical free margines on femoral nerve were 2 cm from the tumor (Figure 1). We did not identify any postoperative surgical complications, except complications in left femoral nerve transection (quadriceps muscle weakness and wasting, loss of knee jerk and numbness along the medial side of the thigh and anteromedial side of the calf).

Definitive HP analysis revealed epithelioid sarcoma with high mitotic feature with free tumor margins (minimally 0.3 cm). HP analysis also revealed tumor metastasis in lymph nodes (3/4) with lymphovascular invasion. The tumor stained highly positive for anti-pan cytokeratin antibody (AE1/AE3), vimentin, cancer antigen 125 (CA125), anti-cytokeratin antibody (MNF116), hematopoietic progenitor cell antigen (CD34) and epithelial membrane antigen (EMA) markers. Calretinin, transformation-related protein 63 (p63), Wilms tumor protein (WT1), cytokeratin 5/6, chromogranin, MART1, cytokeratin 20, factor VIII, CD68 were negatively stained and CEA (Figure 2) was moderately stained. We did not perform staining for integrase interactor 1 (INI1) and SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1(SAMARCB$_1$). Additional imaging procedures, positron emission tomography – computed tomography (PET–CT), that we performed after the surgery, did not reveal any distant metastasis.

The case was then reviewed at tumor board and after analysis of HP and imaging findings, it was decided that the patient should recived conformal radiotherapy without adjuvant chemotherapy. The patient was followed with close surveillance. Since then, the patient was seen every month. Six months postoperatively, there were no evidence of local relapses and distant metastases.

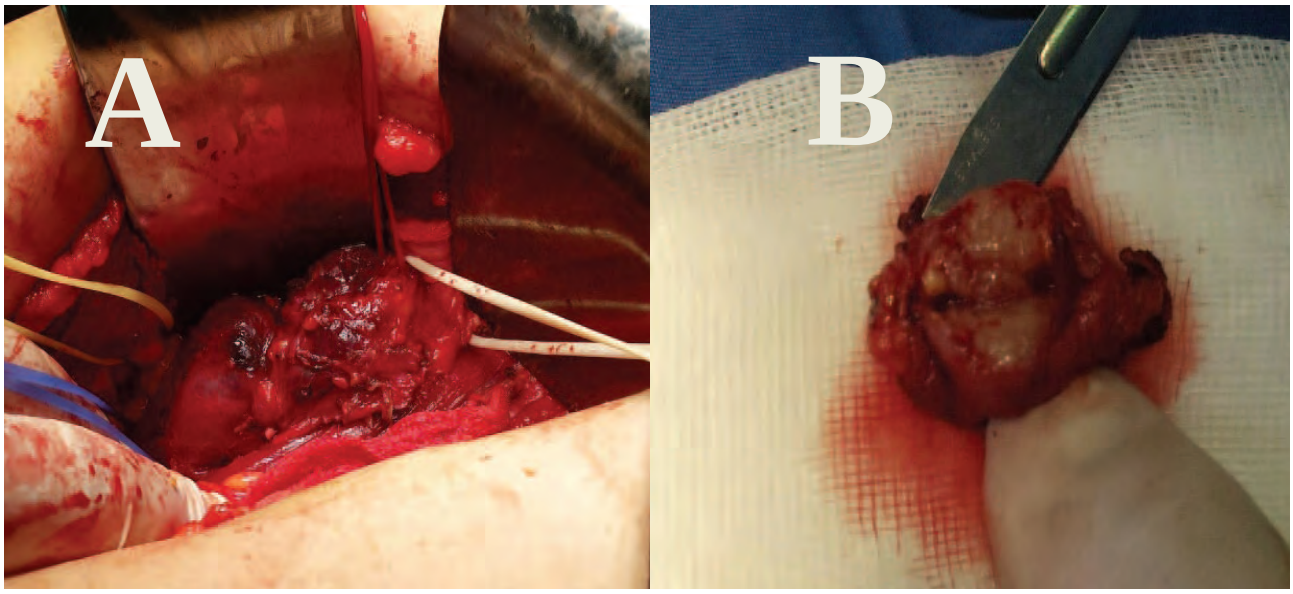

Fig. 1 – Intraoperative findings and tumor after excision.
A) White and blue string mark free margins of left femoral nerve; B) Tumor after excision.

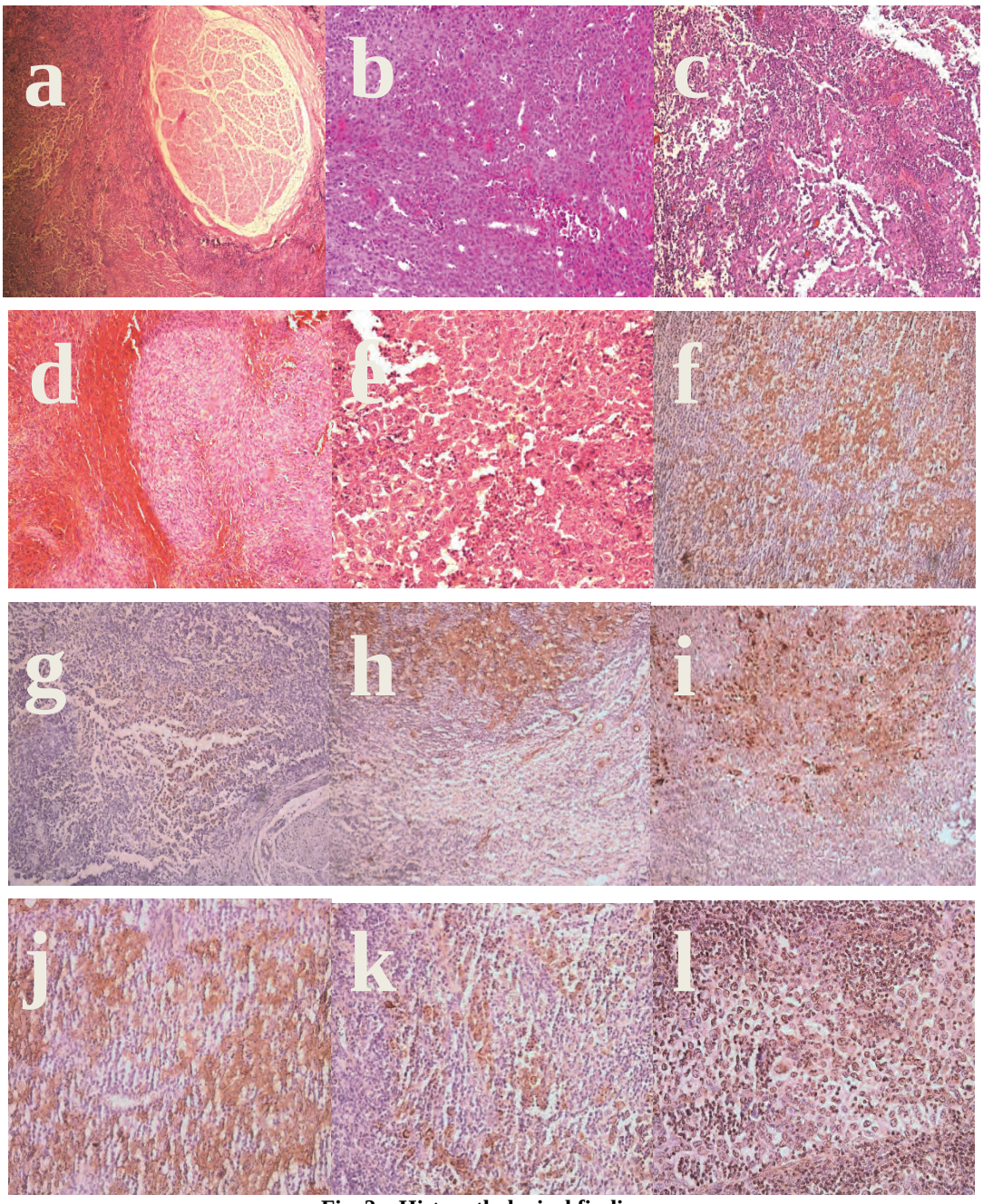

Fig. 2 – Histopathological findings.
a) Hematoxylin and eosin stain (H&E), 5 x 10; b) H&E, 10 x 10; c) H&E, 10 x 10; d) H&E, 10 x 10; e) H&E, 20 x 10; f) Positive staining for AE1/AE3 10 x 10; g) Positive staining for CA125 10 x 10; h) Positive staining for CD34 10 x 10; i) Moderate staining for CEA 10 x 10; j) Positive staining for EMA 20 x 10; k) Positive staining for MNF116 20 x 10; l) Positive staining for vimentin 20 x 10.

Discussion

ES is an aggresisive and rare soft tissue tumor, which was first described by Enzinger [6] in 1970, even with currently available therapy. After reviewing the avaible literature data, we did not find any case of ES at femoral nerve, and, as far as we know, it was the first case of ES at this anatomical location.

Differential diagnosis of epithelioid sarcoma from other forms of cancer is challenging and require various immunohistochemical stain analysis [7].

The prognosis of epithelioid sarcoma is very poor, but multimodal treatment is clearly important to raise the average survival rate of patients. The treatment of choice for ES is wide surgical resection and adjuvant chemoradiotherapy with ifosamide and adriamycin, and in some cases gemcitabine/docetaxel for an aggressive recurrent tumor [7–12]. The role and choice of adjuvant hemiotherapy still remains unclear because it was rare disease [9]. Patients who have had adjuvant radiation therapy show lower recurrence rates [9–11].

It was reported that five years of overal survival (OS) for ES after surgery and chemoirradiaton was 32–78% [8]. OS depends of tumor size, local recurrence (proximal location has poorer prognosis then distal), lymph node involvement, number of mitoses, tumor necrosis, hemorrhage and vascular invasion [1, 8–11]. ES have a high rate of distant metastases especially in lungs and lymph nodes, ranging from 40% to 57% [1, 2, 7, 10, 11].

Development of target antibody therapy might provide alternative therapeutic options. Thway et al. [12] found that inactivation of SMARCB1/IN1 (tumor suppressor gene) played a crucial role in tumorogenesis of ES. Immunochemical studies revealed that 85% to 93% of cases have inactivated SMARCB1/IN1 and target therapy restoring SMARCB1/IN1 gene function could provide new therapy in ES treatment.

There is still a question about prophylactic lymph node dissection for the ES patients. Curent data suggest that lymph node dissection is indicated only in cases when lymph node involvement was observed by preoperative diagnostic imaging (MRI, PET-CT). After HP conformation that nodes are positive, we can perform serial lymph node dissection and adjuvant radiation therapy [10].

Conclusion

Our case suggests that early diagnosis of ES with radical wide resection can improve prognosis. Novel strategies are needed to improve the survival of patients with these highly aggressive sarcomas.

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Intracranial aneurysm as extra-renal manifestation of polycystic kidney disease – A case report

Intrakranijalna aneurizma kao ekstrarenalna manifestacija policistične bolesti bubrega

Violeta Rabrenović*†, Slobodan Ćulafić‡, Milorad Rabrenović§, Tamara Dragović†‖, Saša Trešnjić‡, Siniša Mašić¶, Radomir Matunović†, Svetlana Antić*, Milica Petrović*, Dejan Pilčević*†, Aleksandar Rakonjac††**

Military Medical Academy, *Clinic of Nephrology, §Center for Hyperbaric Medicine, ‖Clinic of Endocrinology, ¶Institute of Hygiene, **Clinic of Cardiology, ††Institute of Radiology, Belgrade, Serbia; University of Defence †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‡Special Hospital St. Sava, Belgrade, Serbia

Abstract

Introduction. Polycystic kidney disease is a hereditary kidney disease characterized by the occurrence of cysts (fluid-filled enlargements) in cortex or medula of the kidney, and is inherited in an autosomal dominant or autosomal recessive manner. In addition to multiple cysts in kidneys, there may be many extra-renal manifestations (cysts of the liver, pancreas, lungs, heart, etc.), among which the most serious one is intracranial aneurysms. **Case report.** A 57-year-old female patient with polycystic kidney disease and stage IV renal failure was hospitalized at our clinic due to decreased renal function, the development of urinary tract infections, headaches and unregulated blood pressure despite the usual treatment. This patient also had a number of associated diseases: obesity, diabetes mellitus (the insulin-dependent type), hypothyroidism, and depression syndrome. After better regulation of blood pressure, resolved urinary tract infections and improved renal function, there were still persistent headaches (resulting in the excessive use of analgesics). With adequate preparation, multislice computed tomography (MSCT) angiography of blood vessels of the head was performed. As a result, we diagnosed the saccular intracranial aneurysm (IA) with anterior localization. Regarding the symptoms, age and comorbidity, digital subtraction angiography (DSA) was performed, and showed saccular IA (5.2 mm × 4 mm), with wide neck affecting both middle cerebral artery branches (MCA). During the procedure the stent was placed, which filled the aneurysm with spirals, cutting it off from circulation. After the successful procedure and without further complications, the patient no longer had headaches and blood pressure was maintained within the required limits with stable parameters of chronic renal failure. **Conclusion.** The case of the patient with polycystic kidney disease, stage IV chronic renal failure, with a number of comorbidities (headache, obesity, hypertension, diabetes mellitus, hypothyroidism) and diagnosed with symptomatic intracranial aneurysm was successfully solved with a multidisciplinary approach, emphasizing the importance of teamwork in daily practice.

Key words:
polycystic kidney disease; kidney failure, chronic; intracranial aneurysm; diagnosis; angiography, digital subtraction; stents; treatment outcome.

Apstrakt

Uvod. Policistična bolest bubrega je oboljenje koje karakteriše pojava cista (proširenja ispunjenih tečnošću) u kori ili srži bubrega, a nasleđuje se autozomno dominantno ili autozomno recesivno. Osim multiplih cista u bubrezima, mogu se javiti i mnoge ekstra-renalne manifestacije (ciste jetre, pankreasa, pluća, srca, itd) među kojima je najozbiljnija pojava intrakranijalnih aneurizmi. **Prikaz bolesnice.** Bolesnica u dobi od 57 godina sa policističnom bolešću bubrega i hroničnom bubrežnom slabošću – IV stadijum, primljena je u našu ustanovu zbog pogoršanja bubrežne funkcije, razvoja urinarne infekcije, pojave glavobolja i neregulisanog arterijskog pritiska i pored redovne terapije. Inače, kod bolesnice su bile prisutne i pridružene bolesti: gojaznost, dijabetes melitus (insulin-zavisan tip), hipotireoza, depresivni sindrom. Nakon bolje regulacije arterijskog pritiska, sani-

Correspondence to: Rabrenović Violeta, Military Medical Academy, Clinic of Nephrology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: violettarab@gmail.com

ranja urinarne infekcije, poboljšanja parametara bubrežne funkcije, zbog upornih glavobolja (zbog kojih je prekomerno koristila analgetike), uz odgovarajuću pripremu učinjena je *Multislice Computed Tomography* (MSCT) angiografija krvnih sudova glave, kojom je dijagnostikovana sakularna intrakranijalna aneurizma anteriorne lokalizacije. Obzirom na simptomatologiju, godine i komorbiditete, a u cilju dalje dijagnostike i lečenja učinjena je digitalna subtrakciona angiografija (DSA) u toku koje je zapažena sakularna aneurizma širokog vrata dimenzija 5,2 × 4 mm iz koje izlaze obe grane ACM. U toku procedure plasiran je stent, kojim je aneurizma ispunjena spiralama i isključena iz cirkulacije. Procedura je protekla bez komplikacija, a nakon toga bolesnica nije imala glavobolje, arterijski pritisak se održavao u zadovoljavajućim granicama, uz parametre hronične bubrežne slabosti koji su bili stabilni. **Zaključak.** Prikaz bolesnice sa policističnom bolešću bubrega, hroničnom bubreznom slabošću – stadijum IV, sa brojnim komorbiditetima (gojaznost, arterijska hipertenzija, dijabetes melitus, hipotireoza) kod koje je dijagnostikovana simptomatska intrakranijalna aneurizma, uspešno je rešena multidisciplinarnim pristupom, što naglašava značaj timskog rada u svakodnevnoj praksi.

Ključne reči:
bubreg, policistična bolest; bubreg, hronična insuficijencija; mozak, aneurizma; dijagnoza; angiografija, digitalna suptrakciona; stentovi; lečenje, ishod.

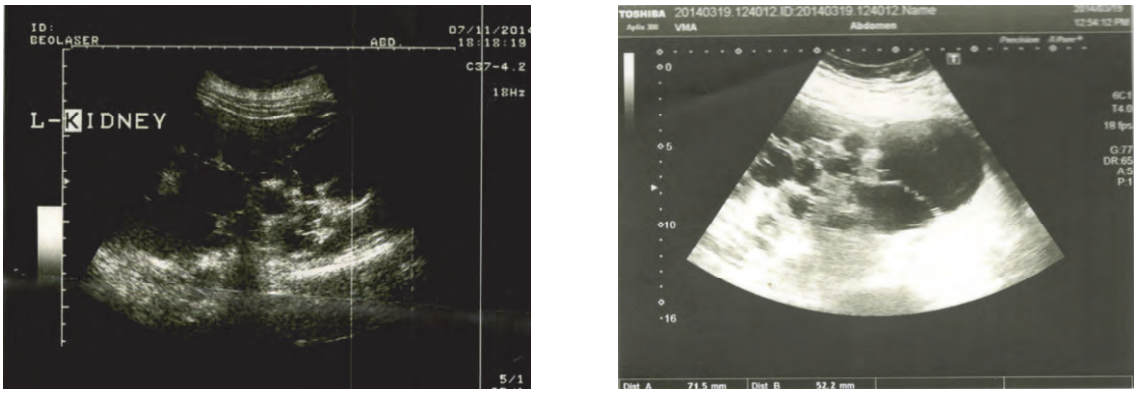
Introduction

Polycystic kidney disease is an inherited kidney disease that is characterized by the occurrence of cysts localized in the cortex or medulla of the kidney [1, 2]. This disease is inherited as an autosomal dominant (incidence 1: 500 to 1,000) or autosomal recessive one (incidence 1: 6,000 to 40,000), which determines the clinical manifestation and prognosis [3, 4]. The progression of autosomal dominant polycystic kidney disease (ADPKD) leads to a decrease in renal function and development of chronic renal failure, with further progress towards the terminal stage [1, 2]. In addition to multiple cysts in the kidneys, there are extra-renal manifestations of ADPKD in the form of cysts of other organs (liver, pancreas, lungs, spleen, brain, etc.) [1, 2]. In 4%–41.2% of patients, one of the most severe complications of the disease is the occurrence of intracranial aneurysms (IA) [2, 5, 6]. IA are usually localized in the anterior circulation, and opinions about their size and the manner of monitoring and methods of treatment are divided [5–7]. Also, there is no standardized protocol for screening of intracranial aneurysms in patients with ADPKD [6]. Some authors believe that there is a minimal risk of rupture of asymptomatic aneurysms of 5–7 mm in size, while other authors believe that there is a risk of rupture even with small aneurysms [7–11].

Case report

A 57-year-old female patient was admitted to the hospital due to worsening of chronic renal failure as a result of polycystic kidney disease, the development of urinary tract infections, intense headaches and high blood pressure. Since the age of 35 she had been treated for polycystic kidney disease, chronic kidney failure (serum creatinine 130 μmol/L) and hypertension and she had occasionally been treated by a nephrologist. Three months before hospitalization, she started experiencing frequent headaches, and she was taking analgesics every day. Her blood pressure was not regulated satisfactorily, despite antihypertensive therapy (max. value was 190/105 mmHg). During the outpatient visit, the physician observed serum creatinine 294 μmol/L, urea 21.7 mmol/L, glomerular filtration rate (GFR) [Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)] 15.2 mL/min/1.73 m^2, and recommended hospitalization. Other observed conditions were hypothyroidism (on substitution therapy with levothyroxine sodium), diabetes mellitus – insulin dependent type, obesity, and depressive syndrome. Also, there was family history of polycystic kidney disease. In addition to obesity of general type [body mass index (BMI) 40.51 kg/m^2] objective examination revealed light abdominal tenderness on deeper palpation (palpated enlarged kidneys) while other findings were regular. Laboratory analysis showed: erithrocyte sedimentation rate (ESR) 83 mm/h, serum urea 20.0 mmol/L, serum creatinine 208 μmol/L, and in the urine sediment: leukocyturia with a positive urine culture at *Escherichia colli* > 100,000 CFU/mL, GFR (CKD-EPI) 22.2 mL/min/1.73 m^2. The kidney ultrasound showed both kidneys enlarged (> 18 × 7.3 cm, with echogenic parenchyma to 1.2 cm), cystic changes with the largest cyst in the right kidney 7.1 × 5.3 cm, and 5.1 cm in the left kidney, nephrolithiasis and without hydronephrosis (Figure 1). After the antibiotic treatment, according to the findings of urine culture and bacterial sensitivity, the infection was resolved, the parameters of inflammation were normalized along with the stabilization of parameters of azotemia (serum creatinine 193 μmol/L). The blood pressure was regulated by adjusting antihypertensive medication. In order to examine persistent and intense headaches and through the further exploration of polycystic disease (after patient preparation for a contrast examination), multislice computed tomography (MSCT) angiography of the head with the blood vessels of the brain was performed. The image revealed a saccular aneurysm in the area of the right branch of middle cerebral artery (MCA). Other images of the arterial blood vessels of the head did not reveal changes. In the interest of further diagnosis and treatment, digital-subtraction angiography (SA) was performed, revealing saccular aneurysm (5.2 × 4 mm) in the area of MCA branch, with wide neck, encompassing both MCA branches (Figures 2 and 3). During the procedure, a stent was placed in the neck of the MCA aneurysm, which was filled with spirals and the aneurysm was cut off from circulation (Figure 4). The procedure was performed without

complications, thus eliminating headaches and the need for excessive analgesic use. Arterial pressure was maintained within the required limits and parameters of chronic renal failure were stable. During the follow-up examination after 18 months, the headaches had not reoccurred, arterial pressure was maintained within the required limits, and laboratory analysis showed: serum creatinine 264 umol/L, serum urea 19.2 mmol/L, and GFR (CKD-EPI) of 17.1 mL/ min/1.73 m^2.



a) b)

Fig. 1 – Renal ultrasound of polycystic changed kidneys: a) left and b) right. Several cysts in both kidneys, which are changed and enlarged.

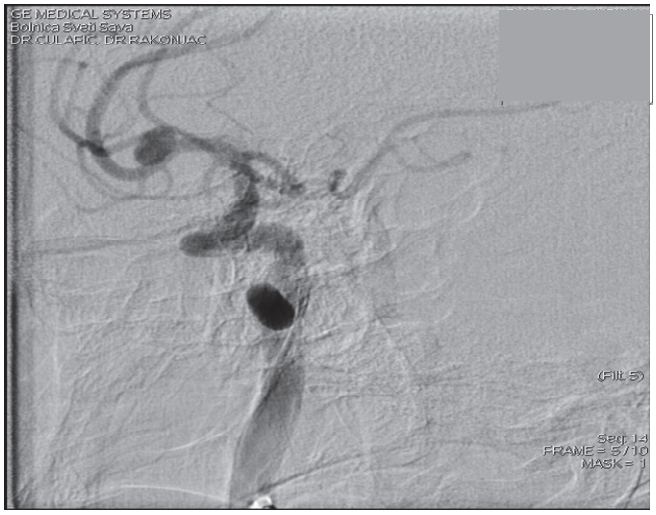


Fig. 2 – Digital subraction angiography (DSA) of right carotid arthery, showing saccular aneurysm in the region of midle cerebral artery (MCA) branch, with wide neck, affecting both MCA branches.

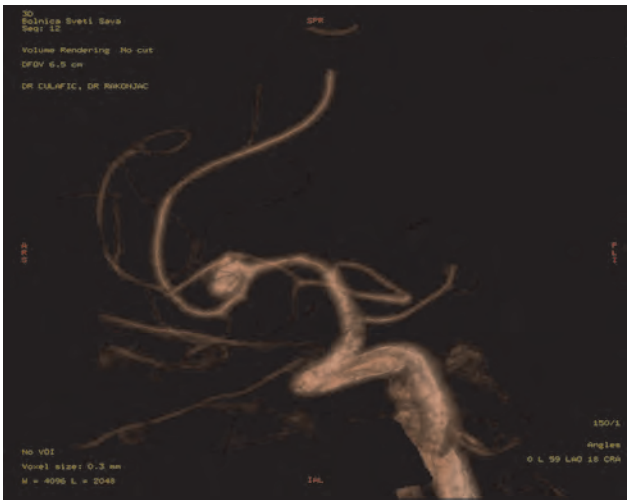


Fig. 3 – 3D angiography of right carotid arthery confirming aneurysm of right branch of middle cerebral artery (MCA) with both branches exiting.

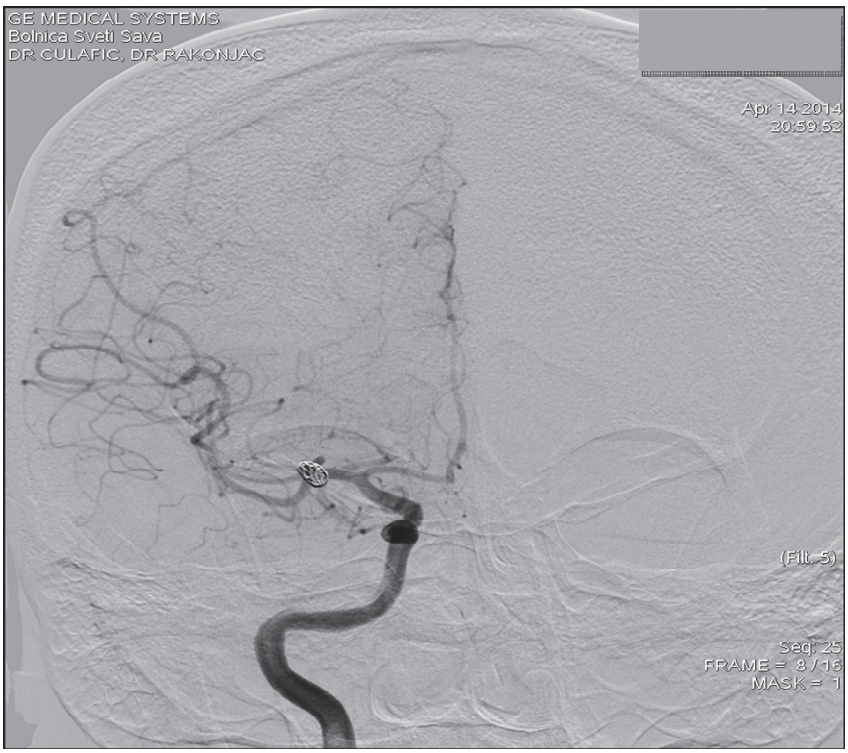


Fig. 4 – Digital subfraction agiography (DSA) of right internal carotid artery (ACI) showing stent placed in the neck of aneurysm of middle cerebral artery (MCA), filled with spirals and cut-off from circulation.

Discussion

ADPKD is the fourth most frequent renal disease with progression leading to terminal renal failure [12]. However, this disease is characterized by severe extrarenal manifestations, one of which is the appearance of IA. Their formation in ADPKD is linked to mutation of polycystin-1 (PKD1) and polycystin-2 (PKD2) [13, 14]. It is believed that the expression of these two proteins in the endothelium and smooth muscle of blood vessels and their reduced level, observed under experimental conditions, are responsible for the development of IA [14]. The incidence of IA in ADPKD is 4–7 times higher than in the general population, more frequent (22%) in patients who have a family history of IA or cerebral bleeding [5, 15]. Asymptomatic IA in an ADPKD occur with a prevalence of 9.3%, while unruptured aneurysms in patients with a family history of IA is observed in 21.2% [8]. The most serious complication is rupture of IA representing life-threatening complication. In some studies, it is stated that the average age of patients with ADPKD and ruptured aneurysm is 41, while in the general population, the average age of ruptured IA is 51 years [15, 16]. Also, many authors note that the risk of ruptured IA increases with age, and describe the prevalence of 23.3% in those older than 60 [15, 16]. For this reason, Niemczyk et al. [17] recommend that the screening examination of the head and the blood vessels of the brain should be performed in patients with ADPKD older than 45 years.

Our patient, who had a family history of polycystic kidney disease, developed symptoms of renal failure and hypertension at the age of 35. IA was discovered 22 years later, after the onset of headaches and arterial hypertension, which had not been sufficiently regulated with therapy.

Many authors suggest that uncontrolled hypertension with ADPKD is an additional risk of complications. The impact of hypertension on the occurrence IA or aneurysm rupture was the subject matter of many studies, and opinions are divided. Some authors believe that hypertension is a risk factor for rupture and massive subarachnoid hemorrhage, and others have found that arterial hypertension in ADPKD represents only indication for screening tests in the vicinity of IA, especially in patients older than 45 years [18–20]. According to Kulesza et al. [20], risk is most associated with the duration of hypertension.

Headaches may represent neurological symptoms, which is associated with the occurrence of IA, and also may precede subarachnoid hemorrhage. Niemczyk et al. [21] report previous occurrence of headache as the most common neurological manifestations in 63% of patients with ADPKD who had IA and cerebral hemorrhage. They also noted that in this group of patients, hypertension was present in 63%, and that 38% had data related to the occurrence of family history of subarachnoid hemorrhage [21].

The occurrence of headaches in our patient and the excessive use of analgesics, along with several years of arterial represented a risk for the long-term progression of chronic renal failure. Tests revealed intracranial aneurysm, which was symptomatic and associated with arterial hypertension. Special precautions were used in preparation for recording contrast, bearing in mind that this was a patient with stage IV chronic kidney disease.

Many authors have an opinion that in patients with ADPKD, the size of the aneurysm is the leading risk factor [22]. Thus, Morita et al. [23] described 6.697 intracranial aneurysms that were discovered by accident in 91% of patients. They observed that the aneurysm > 3 mm had an annual rupture rate of 0.95%, and the risk is increased with increasing size of the aneurysms, especially for those with diameter IA > 7 mm. Results of some studies indicate that in IA larger than 5 mm there is a risk of increase, and in IA larger than 7–10 mm there is a high risk for rupture [7]. A group of Chinese authors described that in IA smaller than 5 mm rupture was observed in 50.9% of patients, but also that rupture of of 2–5 mm was observed in 47.2 % of patients [24].

Our patient had an aneurysm of 5.2 mm, a medium-sized IA according to Liu et al. [24], which was symptomatic and required the appropriate treatment.

Intracranial aneurysms in ADPKD are most often (more than 90% of cases) anterior localized (internal *a. carotis*, a. *cerebri media, a. comunicans anterior, a. cerebri anterior*), and can sometimes be multiple (18%–31%), while other localizations (such as the posterior) are rare [6, 15, 17, 25]. In addition, the shape of the aneurysm is most frequently saccular intracranial, while fusiform ones are less frequent [15].

Our patient had a saccular aneurysm that was anterior localized, and bearing in mind the accompanying headaches, increased blood pressure and other co-morbidities (obesity, diabetes mellitus, hypothyroidism, depressive syndrome) it was decided to carry out the treatment of endovascular stent implantation. Today IA is treated by endovascular or standard neurosurgical methods, and the primary goal is to prevent subarachnoid hemorrhage [26, 27]. When comparing these methods, Quresh et al. [26] concluded that surgical treatment of ruptured IA has advantages compared to endovascular methods, because the obliteration rate of the aneurysm is higher and the need for additional treatment decreases. However, the one-year survival rate was better in the group treated with endovascular methods. They pointed out that the clinical outcome was not impacted by the need for retreatment or incomplete obliteration during the endovascular treatment. According to many authors, in the treatment of unruptured aneurysms, endovascular treatment should be the method of choice, because of a good outcome and shorter hospitalization [26, 27]. It is particularly recommended in patients with unruptured aneurysms who have associated comorbidities, weaker clinical status, or in case when they are older than 65 years [27–29].

Conclusion

A case of a female patient with polycystic kidney disease, stage IV chronic kidney failure, a number of co-morbidities (obesity, hypertension, diabetes mellitus, hypothyreoidism) and diagnosed with symptomatic intracranial aneurysm which was successfully resolved, demonstrates the importance of a multidisciplinary approach and stresses the importance of teamwork in daily practice.

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LETTER TO THE EDITOR
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Glaucoma Weeks and Glaucoma Screening/Prevention

Nedelje glaukoma i skrining/prevencija glaukoma

To the Editor:

Glaucoma is the second leading cause of blindness in the world and represents significant social and health problem [1]. Primary open angle glaucoma (POAG) is the most common type of the disease affecting more than 60 million people worldwide [2]. About 2% of population over 40 years of age has POAG, and this percentage increases to ~4% at the age of 80 years [3]. The first sign of visual loss can be detected when 35% of retinal ganglion cells have already been lost [4]. There is no available method that reliably predicts glaucoma before appearance of the disease and today it is not diagnosed at the percentage in which it is actually present in the population (about half of the individuals remain undetected until the terminal stages of the disease) [5,6]. Therefore, it is particularly important to prevent the disease, its progression and loss of vision.

According to this, 10 years ago we started the national public awareness campaign on glaucoma during the World Glaucoma Week which takes place each year in March within the period of five days. The World Glaucoma Week is a collaborative project between the World Glaucoma Association and the World Glaucoma Patient Association which contributes to the elimination of glaucoma blindness by alerting people to have regular eye checks. In Serbia, eye checks have been realized at the Clinic of Ophthalmology (its outpatient department) of the Clinical Centre Kragujevac in cooperation with the Association of the Glaucomatologists of Serbia. Within the Glaucoma Week, Glaucoma Weeks for preventing/screening glaucoma a free ophthalmic examinations are available to all interested persons. Examinations include slit-lamp examination, measurement of intraocular pressure (IOP) and evaluation of the cup/disk ratio of both eyes as well as a medical history taking. The diagnosis of glaucoma is confirmed by a glaucoma specialist based on the findings obtained. The POAG is defined with elevated IOP and/or glaucomatous disc changes, typical glaucomatous field defects, an open iridicorneal angle and with no secondary causes. Elevated IOP with no optic disc changes and no visual field defects with open iridocorneal angle is considered as ocular hypertension. The results are considered as positive if IOP is > 21 mm Hg and the cup/disk ratio > 5. During observations, family history of glaucoma is also marked for each person.

In the period observed (2008–2017) glaucoma screening program involved 1,392 persons, predominantly women (n = 907) and adults aged 50–84 years (n = 1,030) (Table 1). The results showed that the number of people screened every year was increasing, from 79 in 2008 to 270 in 2017 (3.5-fold increase). The highest increase was observed in 2017 for men and persons aged 15–49 years.

Table 1

Number of persons by gender and age screened for glaucoma during Glaucoma Weeks in previous 10 years (2008–2017)

Year	Total persons	Gender		Age (years)	
		female	male	15–49	50–84
2008	79	52	27	10	69
2009	96	64	32	16	80
2010	101	79	22	16	85
2011	108	87	21	17	91
2012	127	99	28	23	104
2013	131	99	32	22	109
2014	140	100	40	22	118
2015	151	101	50	40	111
2016	182	103	79	49	133
2017	277	123	154	147	130
Total	1,392	907	485	362	1,030

Detailed analysis of the results obtained in last 5 years (2013–2017) showed that IOP of screened persons was between 10 and 40 mmHg. The values of IOP < 21 mmHg and c/d ratio < 0.5 were found in 662 (75.14%) persons suggesting that they had no glaucoma. The IOP greater than 21 mm Hg was measured in 163 (18.5%) patients. The IOP greater than 24 mm Hg was found in 56 (6.36%) patients. The ratio c/d ≥ 0.5 was found in 219 (24.86%) patients, while in 351 (39.84%) patients the c/d ratio was ≥ 0.7 confirming the existence of POAG (Table 2). Thus, 163 were suspected of having glaucoma and 56 patients were diagnosed with glaucoma in accordance with measured IOP. IOP values in combination with c/d ratio excluded a total of 530 screening individuals on glaucoma. The remaining 351 patients were taught about the nature of the disease and how to live with it.

Correspondence to: Mirjana Janićijević Petrović, Clinic of Ophthalmology, Clinical Centre of Kragujevac and Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34 000 Kragujevac, Serbia. Phone: +38134/369828. E-mail: mira.andreja@yahoo.com

Table 2
Prevalence of normal/elevated intraocular pressure (IOP) and enlarged cupping (performance) of screened persons (n = 881) in last 5 years (2013–2017)

Paramaters	Number (%) of persons
IOP < 21 mmHg; cupping < 0.5	662 (75.14)
IOP ≥ 21 mmHg	163 (18.5)
IOP ≥ 24 mmHg	56 (6.36)
C/D ≥ 0.5	530 (60.16)
C/D ≥ 0.7	351 (39.84)
IOP ≥ 21 mmHg; cupping ≥ 0.5	219 (24.86)

C/D – cup/disk ratio.

Positive family glaucoma disease was detected in 308 (35%) screened persons (in 30 and 279 persons who belonged to younger and older group, respectively).

Statistically significantly higher number of persons with elevated IOP had positive family history of glaucoma. There was also statistically significantly higher number of patients with pathological c/d ratio of positive family history of glaucoma in relation to number of persons with lower values of IOP and normal c/d ratio and positive family history of glaucoma ($p = 0.026$; $p = 0.08$, respectively). Myopia associated with glaucoma and positive and negative family history of glaucoma was detected in 15 and 23 persons in the younger age group, respectively, while in the older group there were 37 such persons with postive family history of glaucoma and 52 persons with negative family glaucoma.

Statistically significant number of persons was presented with elevated IOP, myopia and glaucoma as well as statistically significant number of patients with pathological c/d ratio, myopia and glaucoma in relation to a number of persons with lower values IOP/normal c/d ratio, myopia and glaucoma ($p = 0.012$; $p = 0.022$, respectively).

Number of newly registered patients in an initial stage of glaucoma in relation to number of newly discovered in the terminal stage of disease significantly increased, especially in last 5 years of the Glaucioma Week program in our contry. Today, in addition to reducing number of people suffering from progressive glaucoma, number of newly discovered and controlled glaucoma increased, without rapid progression or any progression of the disease. Our glaucoma program has greatly contributed to about one half of reduction in incidence of terminal glaucoma. We want to point out that the implementation of this screening program in our country was expected to show a positive trend resulting in early detection of the disease and consenquently providing timely treatment, reduction of glaucoma progression and, finally, reduction of loss of vision in our patients.

Katarina Janićijević[*], Tatjana Šarenac Vulović[†‡], Sanja Kocić[*], Snežana Radovanović[*], Svetlana Radević[*], Mirjana Janićijević Petrović[† ‡]

University of Kragujevac, Faculty of Medical Sciences, [*]Department of Social Medicine, [†]Department of Ophthalmology, Kragujevac, Serbia; Clinical Centre of Kragujevac, [‡]Clinic of Ophthalmology, Kragujevac, Serbia

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LETTER TO THE EDITOR
(RESEARCH LETTER)

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Impedance aggregometry may help in prediction of increased perioperative bleeding in cardiac surgery

Impedantna agregometrija može pomoći u predviđanju povećanog perioperativnog krvarenja u kardiohirurgiji

To the Editor:

Preoperative risk assessment for increased perioperative bleeding is essential for surgical patients, and, especially for cardiac surgery patients. Specificity of operations on open heart, does not just reflect in complexity of surgical action and usage of extracorporeal bloodstream which disturbs the hemostasis, but also in the fact that the patients often are preoperatively treated with antiplatelet drugs [1]. The standard preoperative hemostasis testing, including prothrombin time (PT, INR) and activated partial thromboplastin time (aPTT), does not give us an insight into what is happening with platelets which are one of the main factors of normal hemostasis.

Impaired platelet function is rarely congenital; the most often it is acquired, commonly caused by medications such as acetylsalicylic acid (ASA) and other antiplatelet drugs. While ASA usefulness in decreasing adverse ischemic events in patients with coronary artery disease is clearly proven, there are different views about preoperative stopping of ASA and its connection with increased risk of bleeding during cardiac surgery operations [2].

Preoperative ASA therapy, is forbidden 5–7 days before cardiac surgery intervention by recommendations in order to reduce the potential risks of bleeding during operation. However, without testing platelet function we cannot know whether it is disrupted or whether there is a presence of residual antithrombotic agent therapeutic effect, especially taking into account that each person individually respond to the therapy [3]. In this sense, impedance platelet aggregometry as the method for precise assessment of platelet function may provide valuable information on platelet function and consequently help in estimating the risk of increased bleeding and transfusion requirements. For this purpose, semi-automatic impedance aggregometer (Multiplate analyzer, Dynabyte Medical, Germany) is commonly used. It analyzes platelet function from the whole blood sample. One of the tests that is performed by this apparatus is the ASPI test which uses arachidonic acid as a substrate of platelet cyclooxygenase. When cyclooxygenase is blocked, thromboxane formation is inhibited and, in turn, platelets activation. The ASPI test is sensitive to cyclooxygenase-blockers such as ASA.

We did not find in literature a strong recommendation for cut-off values of the ASPI test that represents moderate or high risk for increased bleeding during and after cardiac surgery operation. On the other hand, there are many recommendations for values of the ADP test which demonstrates residual antiplatelet effects of the ADP blockers such as clopidogrel [4].

Some researchers suggest that aggregometry prior to cardiac surgery for detection of residual antiplatelet effect of ASA is unnecessary and that preoperative ASA increases postoperative bleeding, but this may be avoided by the use of the ASA doses < 325 mg/day [3]. On the other hand, some researchers suggest individual approach for each patient going to heart surgery by determining platelet aggregability and stopping ASA [4,5].

Petricevic et al. [6] in their study, which included 101 elective cardiac surgery patients, demonstrated significant negative correlation between platelet function measured with ASPI test and increased bleeding. A conclusion [7] of the study performed by Myles et al. was that ASA had to be stopped before cardiac surgery because of the bleeding complications. Preoperative administration of ASA is connected with higher blood loos during surgery without positive effect on graft patency in comparison with taking ASA 6h after operation. Aspirin increases postoperative blood drainage after cardiac surgery and also a need for blood transfusions [8].

Recently published Guidelines on perioperative medication in adult cardiac surgery [11] have suggested that in patients on ASA who needed to undergo coronary artery bypass graft (CABG) surgery continuing ASA thoroughout the preoperative period should be considered. However, in patients who refused transfusion, who are at high risk of re-exploration for bleeding such as complex and redo opeartions, with severe renal insufficinecy, haematological diseases and herediraty platelet function deficinecy, stopping ASA at least 5 days before surgery was recommended (class IIa, level C of recommendation).

Having in mind these opposite attitudes, we wanted to established whether preoperative platelet function testing for

Correspondence to: : Milan Lazarević, MD, Clinic for Cardiac Surgery, Clinical Center Niš, Niš, Serbia, Bulevar dr Zorana Đinđića 48, 18 000 Niš, Serbia. E-mail: dr_m.lazarevic@hotmail.com

the presence of residual antiplatelet effect of ASA before cardiac surgery, even after stopping ASA at least 5 days before surgery, may provide clinically important information that would be related to increase in intraoperative bleeding and consequent increase in blood transfusions. We retrospectively analyzed 60 operated on cardiac surgery patients (double and triple by-pass grafting), who had similar demographic characteristics, the same preoperative antiplatelet therapy (they all received monotherapy with 100 mg of ASA that was ceased 5 days before operation), similar platelet count, length of extracorporeal circulation procedure, coagulation screening, etc. (Table 1).

Table 1

Basic demographic and clinical data of 60 patients enrolled in the study

Data	Group with NPF, n = 31 (51.7%)	Group with IPF n = 29 (48.3%)	*p* value
Gender (female / male), %	25.8 / 74.2	24.1 / 75.8	
Age (years), mean ± SD	65.1 ± 7.31	62.3 ±7.38	> 0.05
Eritrocytes (n x 10^{12}/L), mean ± SD	4.46 ± 0.44	4.37 ± 0.41	> 0.05
Platelets (n x 10^{9}/L), mean ± SD	242.9 ± 60.83	221.7 ± 45.85	> 0.05
Received ASA (% of patients)	100	100	
ASPI test (AU min), mean ± SD	1.056 ± 229.44	416.7 ± 195.95	< .0001
TRAP test (AU min), mean ± SD	1,451.6 ±170.47	1,348.3 ± 259.22	> 0.05
CABG II / CABG III (%)	54.8 / 45.2	51.7 / 48.3	
Perioperative blood loss (mL), mean ± SD	980.6 ± 199.86	1,527.5 ± 637.74	< .0001
Autotransfusion (mL), mean ± SD	388.3 ± 76.07	587.6 ± 210.87	< .0001
Allogeneic transfusion (mL), mean ± SD	180.6 ± 253.53	253.4 ± 294.27	> 0.05

NPF- normal platelet function; IPF impaired platelet function; ASA - acetylsalicylic acid; AU - aggregation unit per minute; SD – standard deviation; CABG – coronary artery bypass graft.

The platelet function testing with the ASPI test was performed in all patients. It was shown that almost 50% of the analyzed patients had impaired platelet function in the ASPI test after cancelling ASA (416.7 ± 195.95 aggregation units *per* minute) which led to increased perioperative drainage for almost 600 mL compared to the group of patients who had unimpaired platelet function (1,527.5 ± 637.74 mL v.s. 980.6 ± 199.86 mL, $p < 0.0001$). Also, the group with the impaired ASPI test received more autologous blood transfusions.

It was shown that preservation of platelet function and the absence of residual antiplatelet effect of acetylsalicylic acid preoperatively resulted in a significantly lower amount of intraoperative bleeding and lower amount of autologous transfusion, which is of great importance for the course and outcome of the surgery.

In conclusion, results of our study showed importance of testing platelet function with the ASPI test before operation, regardless the cessation of the ASA therapy 5 days before elective surgery in order to avoid excessive perioperative blood loss, blood transfusions and to possibly postpone cardiac surgery.

Bearing in mind the complexity of cardiac surgery operations and possible bleeding complications, testing with impedance aggregometry before the intervention should not be ignored. Our data suggest that aggregometry may optimize the timing for surgical procedures, especially in patients who have residual antiplatelet effect.

Milan Lazarević*, Dragan Milić*†, Tomislav Kostic‡, Velimir Perić†, Nenad Jovanović§, Zoran Stanojković‖, Mlađan Golubović‡

Clinical Center Niš, *Clinic for Cardiac Surgery, §Center for Anesthesiology and Reanimatology, ‡Clinic for Cardiology, Niš, Serbia; University of Niš, †Faculty of Medicine, Niš, Serbia; ‖Blood Transfusion Institute, Niš, Serbia

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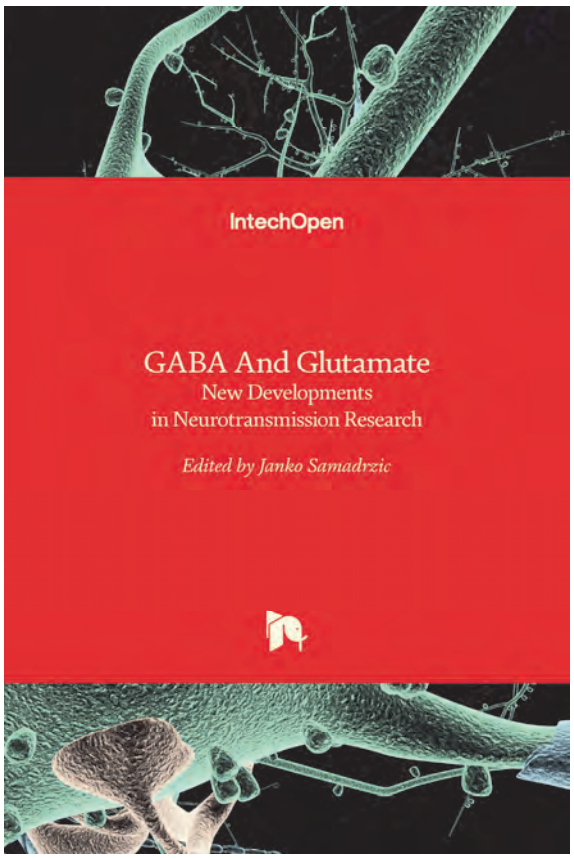
BOOK REVIEW/PRIKAZ KNJIGE

GABA and Glutamate – New Developments in Neurotransmission Research



Title: GABA and Glutamate - New Developments in Neurotransmission Research
Editor: Janko Samardzic
Authors: Janko Samardzic, Dragana Jadzic, Boris Hencic, Jasna Jancic, Dubravka Svob Strac, Gabriela Beatriz Acosta, López-Bayghen Esther, Angulo-Rojo Carla, López-Bayghen Bruno, Hernández-Melchor Dinorah, Ramírez Leticia, Ortega Arturo, Shashi Kant Singh, Ing-Feng Chang, Christiane S. Hampe, Hiroshi Mitoma, Mario Manto, Tina Hinton, Graham A.R. Johnston, B C Hamsini, Bhavana Nagabhushana Reddy, Sankar Neelakantan and Sunitha Palasamudram Kumaran.
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The book “GABA And Glutamate - New Developments In Neurotransmission Research”, edited by Dr. Janko Samardzic, collates the significant contributions of a selected number of neuroscientists that are experienced in the molecular, preclinical, and clinical aspects of neurotransmission research. The seven chapters in this book address the latest research/review data related to GABA/glutamate system's organization and function, the structure of receptors, subtypes and their ligands, as well as the translational approach and clinical implications.

The introductory chapter “GABA/glutamate balance: a key for normal brain functioning” describes the basic function and relevance of GABA/glutamate balance in normal brain, focusing on the role of their receptors. The second chapter, titled “Early life experience: maternal separation, involvement of GABA and glutamate transporters”, summarizes scientific data and opinion regarding maternal separation as a model of early life experience of postnatal stress, with focus on the involvement of GABA and glutamate transporters. The third chapter, “Notch signaling in the astroglial phenotype: relevance to glutamatergic transmission”, addresses issues related to the role of notch signaling in radial glia, with emphasis on glial glutamate transporter regulation as a key element in the molecular mechanisms that support glutamatergic neurotransmission. “Pharmacological studies with specific agonist and antagonist of animal iGluR on root growth in Arabidopsis thaliana” presents original data from a pharmacology-based functional study of ionotropic glutamate receptors (iGluRs) in plants, suggesting a correlation between the putative iGluR-like channel function and the modification of root growth and development in the Arabidopsis roots. This is followed by a very up-to-date review titled “GABA and glutamate: their transmitter role in the CNS and pancreatic islets”, in which the authors address not only the role of both neurotransmitters during development, but also extra-neuronal glutamatergic and GABAergic signaling in pancreatic islets of Langerhans, and possible associations with type 1 diabetes mellitus. Further clinical implications are discussed in the sixth chapter titled “Antagonists of ionotropic receptors for the inhibitory neurotransmitter

GABA: therapeutic indications". The authors examine the antagonism of ionotropic GABA receptors, reflecting on the use of GABA receptor antagonists in the last 10 years and their possible therapeutic potential. Finally, the chapter "Clinical applications of MR spectroscopy (MRS) in neurosciences" delivers a detailed description of the methodology and relevance of MRS as an important diagnostic and research tool in clinical neuroscience.

The key to most complex brain processes lies indeed in the adequate balance between inhibitory and excitatory actions of amino acid neurotransmitters. Furthermore, increases and decreases in their activity are associated with a number of neurological and psychiatric diseases. Therefore, this up-to-date book offers readers a valuable collection of data regarding current and future applications of GABA and glutamate neurotransmission, including promising research strategies and potential clinical benefits.

In addition to the print edition, the book is also freely available online:
https://www.intechopen.com/books/gaba-and-glutamate-new-developments-in-neurotransmission-research

prof. Silva Dobrić
University of Defence
Faculty of Medicine of the Military Medical Academy
Belgrade, Serbia

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d) Conclusion could be a separate chapter or the last paragraph of the discussion;

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Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

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Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

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Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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